ESTABLISHING THE NUTRITIONAL VALUE OF FIELD PEA AS AFFECTED BY FEED PROCESSING AND PEA CULTIVAR FOR POULTRY

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ABSTRACT

The effects of feed processing, pea cultivar and their interaction on the nutritional value of field pea (*Pisum sativum* L.) for poultry were evaluated in regard to its apparent metabolizable energy (**AME_n**), apparent protein digestibility (**APD**), and rate and extent of starch digestion. Amino acid sparing as affected by the rate of starch digestion was studied in laying hens and broiler chickens. Also, the effects of feeding a slowly digested starch (**SDS**) from pea on performance and metabolism of broiler–breeder pullets were investigated.

The first objective of this research was to evaluate the effects of screen–hole size, cold pelleting, and pre–pelleting conditioning temperature on nutrient digestibility of pea. There was no interaction between dietary treatments on all studied parameters. Small hammer–mill screen–hole size (3.2–mm) increased AME_n, APD, and extent of starch digestion values compared with coarse screen–hole size (6.4–mm). The AME_n and extent of protein digestion were not affected by cold pelleting, but the site of protein digestion was affected. In contrast, cold pelleting increased the rate and extent of starch digestion. Pre–pelleting conditioning temperature affected AME_n of pea in a quadratic fashion but had no positive effect on starch digestibility. The 70°C of pre–pelleting conditioning temperature maximized pea AME_n. Increasing pre–pelleting conditioning temperature decreased APD in a linear fashion.

The second objective of this research was to study the effects of feed processing, pea cultivar and their interaction on AME_n, APD, and rate and extent of starch digestion. In vitro and in vivo experiments were conducted. An in vitro procedure simulating the gastric and small intestine conditions of chickens was developed to predict the rate and

extent of starch digestion as affected by pea cultivar and sieve–hole size (0.5–, 1.0–, 2.0–mm). The rate and extent of starch digestion of cereal grain samples (barley, corn, and wheat) was also compared to pea starch. No interactions were found between pea cultivar and sieve–hole size on the kinetics of starch digestion. Pea cultivar affected the rate and extent of starch digestion. The small sieve–hole size in the in vitro assay resulted in a higher rate and extent of starch digestion. Pea starch was slowly digested in comparison with cereal grains. The in vivo experiment confirmed that fine grinding and pelleting improves AME_n and APD. Cultivar effects on AME_n and APD were observed, but no interaction was found between pea cultivar and feed processing.

The third objective of this research was to investigate whether feeding SDS from pea would have sparing effect on amino acid utilization in chickens. In the first experiment, the effects of three levels of pea inclusion 0, 150, 300 g/kg on the response of laying hens to three levels of lysine intake (700, 780, and 860 mg per day) were evaluated using performance and production criteria. This experiment revealed that pea inclusion up to 300 g/kg in laying hen diets was well tolerated by laying hens and improved energy retention as indicated by increased body weight and egg weight. However, this experiment did not confirm the hypothesis that SDS from pea spared amino acids for laying hens. The second experiment investigated the interaction between SDS derived from pea and amino acid levels on the performance and carcass quality of broiler chickens. Six levels of pea inclusion (0, 150, 300, 450, 600, and 750 g/kg) and two levels of amino acids (100 and 85% of Ross × Ross 308 requirement) were examined in a broiler trial (0 – 35 d). The maximum level of pea inclusion recommended in diets increased with broiler age, but the effect of SDS from pea on amino acid sparing could

not be confirmed. In the third experiment, the effects of feeding SDS from pea on growth performance and metabolism of broiler breeder pullets were investigated. Body weight and uniformity of pullets fed pea–based diet were similar to that of a wheat–based diet. Target body weight and uniformity of pullets were not affected by feeding a diet containing 890 g/kg of pea. Mean blood glucose levels and relative liver weight were markedly lower in broiler pullets fed pea–based diet compared with those fed a wheat–based diet.

In conclusion, feed processing independently had a significant effect on the availability of pea nutrients. Pea is a good source of both energy and protein and that it can be partially or completely included to replace wheat and soybean meal in poultry diets. However, the effect of SDS on amino acid sparing could not be confirmed. Further research is needed to examine other feed processing techniques, pea cultivars, level of inclusion, and to understand other metabolism responses to feeding SDS from pea.

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LIST OF ABBREVIATIONS

AA	Amino Acid/s
AIPD	Apparent Ileal Protein Digestibility
AME	Apparent Metabolizable Energy
AME_n	Apparent Metabolizable Energy with Nitrogen Correction
BW	Body Weight
BWG	Body Weight Gain
BMY	Breast Meat Yield
cm	Centimeter
CY	Carcass Yield
D	Dark
d	Day
FCR	Feed Conversion Ratio
FI	Feed Intake
g	Gram
h	Hour
L	Light
m	Meter
RDS	Rapidly Digested Starch
SDS	Slowly Digested Starch
RS	Resistant Starch
SEM	Standard Error of the Mean

1.0. INTRODUCTION

In order to meet the requirements of an increasing world population in the next decade, meat and egg production should be increased dramatically. Therefore, animal feed production will need to increase to supply this future demand. The nutritional value and supply of feedstuffs will also need to be improved. The use of new grain or pulse cultivars with higher nutritional value will also see increased interest, particularly those that grow well in a wide range of environments. Pea is a good candidate for further development in this regard as it can be grown in most temperate places in the world.

The cost of producing poultry meat and eggs is mainly affected by the price of feed ingredients. Poultry feed accounts for 70 to 80% of the production cost. Corn, wheat, and soybean meal are the most common feedstuffs in poultry diets internationally. Corn and wheat are fed mainly as sources of energy and protein, and soybean meal supplies the supplemental protein and amino acids. Soybean, a predominant source of protein for poultry, is a warm season crop and is exported to many areas of poultry production. This impacts the cost of poultry production because of the high price of the meal as well as the additional transportation cost. Field pea (*Pisum sativum* L.) is grown in temperate regions. It has a moderate level of energy and protein that is suitable for use in poultry diets if cost considerations are favorable. The inclusion of pea in poultry feed may replace other expensive feed ingredients and give the feed industry more flexibility in feed formulations.

Field pea or dry pea is grown for both human consumption and animal feed. In Europe, pea is widely used in swine feed (Gatel and Grosjean, 1990). In Western Canada, field pea production has increased rapidly in the last two decades compared to other

crops, such as wheat. However, pea is not used extensively in the Canadian feed industry. Even though pea production in 2010 and 2011 was less than previous years (3.0 and 2.9 MMT, respectively), Canada is the world's largest pea producer and exporter. It supplies approximately 30% of the world production. Moreover, most of field pea is grown in western Canada with 65% of Canada pea's production is produced in Saskatchewan (www.agr.gov.sk.ca).

Feedstuffs are evaluated mainly based on their metabolizable energy and digestible nutrient content. Starch is the main source of dietary energy in poultry diets and supplies more than 50% of the requirement. It is documented that the AME value is well correlated with the amount of digested starch (Wiseman et al., 2000). Starch digestibility is affected by the physical and chemical structures of the starch itself, which varies based on starch origin (Carré, 2004; Wiseman et al., 2006; Singh et al., 2010). Moreover, practical feed processing such as grinding and pelleting that are used commonly in poultry feed mills have been shown to affect starch digestibility (Carré, 2004; Svihus et al., 2005; Abdollahi et al., 2011). However, the effect of processing on pea–based diets in poultry is variable and not well understood because of the many factors that can have an effect, such as particle size reduction, pelleting–conditioning temperature, pea cultivar, and the interaction between pea and other feed ingredients in poultry diets.

In the poultry industry, high production levels and efficient feed conversion are the most important objectives. Therefore, it is critical to formulate poultry diets with balanced and accurate nutrient content for maximum and economical production. In order to achieve these objectives, diets should be formulated with readily digested starch that

can be absorbed in the small intestine. If starch cannot be digested in the small intestine, then it is either fermented by the microflora in the hindgut or excreted. The end products of starch fermentation are volatile fatty acids (**VFAs**) which less energy efficient than glucose for monogastric animals.

In spite of the importance of the extent of starch digestion in poultry nutrition, the rate of starch digestion has been recognized to have an impact on nutrient value. Pea starch is slowly digested and this may benefit animal metabolism and ultimately performance. Feeding a diet with a mixture of starch degradation rates, slow and rapid, might improve poultry performance compared with a diet containing only rapidly digested starch (Weurding et al., 2003a,b). It can be hypothesized that for fast growth of broiler chickens and high egg production of laying hens, starch should be slowly, but completely digested and absorbed in the small intestine.

Overall, this thesis had three strategies. In the first strategy, it was hypothesized that fine grinding size and pelleting would improve pea nutrient digestibility. The hypothesis for the second strategy was that interactions between feed processing and pea cultivar would impact pea nutrient availability. For the third strategy, it was hypothesized that feeding slowly digested starch from pea would affect bird metabolism and enhance poultry performance.

The aim of this thesis was to fill the gap of the limited information on the nutritional value of pea for poultry. The chemical and physical structure of starch, starch digestion in poultry, feed processing, and feeding pea to poultry was first reviewed (**Chapter 2**); the effect of hammer–mill screen–hole size and feed form (mash vs. cold–pellet) on apparent metabolizable energy (**AME_n**), kinetic of starch digestion, and

apparent protein digestibility (**APD**) was studied (**Chapters 3**); the effect of hammer–mill screen–hole size and pre–pelleting conditioning temperature on pea nutritive digestibility was evaluated (**Chapter 4**); the impact of pea cultivar, sieve–hole size, and their interaction on the kinetics of starch degradation (in vitro) was investigated (**Chapter 5**); the interaction between pea cultivar and feed processing on pea nutrient digestibility (in vivo) was examined (**Chapter 6**); the AME, kinetics of starch digestion, and apparent protein digestibility of wheat, corn, and barley were compared (in vitro and in vivo) with pea cultivar (**Chapter 5 and 6**); finally, the effects of feeding slowly digested starch on diet amino acid utilization, poultry performance, and metabolism were investigated (**Chapter 7, 8, and 9**). The results and conclusions from research in Chapters 3 to 9 are discussed and summarized at the end of this thesis (**Chapter 10**).

2.0. LITERATURE REVIEW

This review focuses on starch and its importance in poultry nutrition. Relevant areas include the physical and chemical structure of starch, starch digestibility and its measurement and importance, and factors that affect starch digestibility such as feed processing. Because of its unique starch and starch digestibility characteristics, this review emphasizes pea starch as well as the inclusion of pea in poultry feeding.

2.1. Starch

Starch is the main form of carbohydrate storage in plant seeds. It is a polymer of D–glucose molecules and accumulates in granules, which are different in size and shape among sources (Jane, 2004). Starch is a major nutrient source in feedstuffs such as cereal grains and legumes fed in poultry diets. It supplies more than 50% of metabolizable energy (ME) requirement of poultry flocks. Although starch is a polymer of glucose, its chemical linkages and deposition within seeds vary substantially, and these differences result in variation in the rate and extent of digestion. The end product of starch digestion is glucose, which is the most important metabolite in chicken metabolism and the main energy–yielding substrate.

2.1.1. Chemical Structure of Starch

The empirical hydrated formula of starch is $(C_6 H_{10} O_5 \cdot H_2 O)_n$ and the glycosidic bonds that connect glucose molecules in pure starch are α–glucan (99%). Bonds are α–1,4 and α–1,6 and the frequency of these bonds distinguishes the two different glucose polymers, amylose and amylopectin, found in starch.

2.1.1.1. Amylose

Amylose is a primarily linear polymer of D–glucose and is characterized by relatively few branches (9 to 20 per molecule) with around 99% of the glycosidic bonds being in the α–1,4 form and approximately 1% in the form of α–1,6 bonds (Figure 2.1). The number of branches is mostly related to the size of the molecule (Jane, 2004). Based on its botanical origin, stage of development and extent of processing, the molecular weight of amylose varies between 1×10^5 to 1×10^6 Dalton (Oates, 1997; Buléon et al., 1998). The length of amylose chains ranges from 200 to 700 glucose molecules and the average degree of polymerization (DP) is between 324 to 4,920 glucose molecules (Tester et al., 2004a,b). Native amylose chains form double or single helices. Each amylose molecule has few (2 to 8) non–reducing ends (free –OH on C4) and a reducing end (free –OH on C1). In most starch sources, amylose makes up between 20 to 25% of dry matter. However, some mutant starch sources such as maize contain between 65 to 70% amylose, while others may contain only very small amounts and the latter are termed waxy starches (Parker and Ring, 2001). The difference in size and structure of amylose is based on the origin of starch. The proportion of amylose in round pea starch varies between 33.1 to 48.8% whereas wrinkled pea may contain up to 88% with a DP range from 1000 to 1400 and number of branch points ranging from 2 to 3.2 per molecule (Ratnayake et. al., 2002).

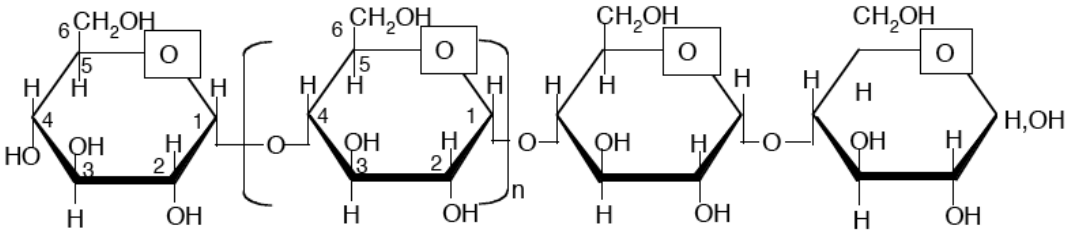

FIGURE 2.1. Amylose structure: Glucose molecules are linked by α–1,4 glycosidic bonds (Taken from Tester et al., 2004b).

2.1.1.2. Amylopectin

Amylopectin is the other fraction of starch (Figure 2.2) and unlike amylose, is highly branched with about 95 and 5% of glycosidic bonds in the α–1,4 and α–1,6 form, respectively (Oates, 1997; Buléon et al., 1998). The molecular weight of amylopectin is much larger than amylose with a range between 1×10^7 to 1×10^9 Dalton (Oates, 1997) and the average of degree of polymerization (DP) is between 9,600 to 15,900 glucose molecules. Each amylopectin chain contains 12 to 120 glucose units so they are shorter than amylose chains (Tester et al., 2004a,b). These chains are linked together by α–1,6 bonds an average of one every 20 glucose molecules (Gallant et al., 1997). The short chains of amylopectin are arranged in clusters linked to the long chain.

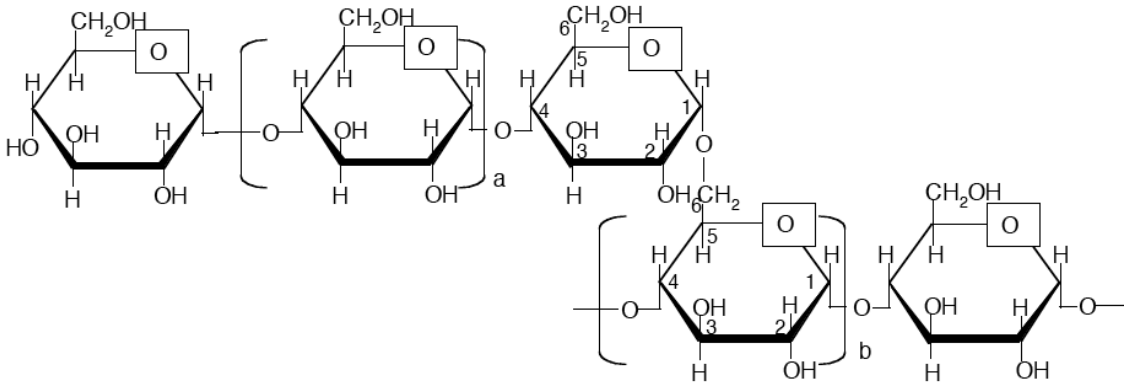

FIGURE 2.2. Amylopectin structure: Glucose molecules are linked by α–1,6 glycosidic bonds at the branching point. a = 12 to 23 glucose units; b = 20 to 120 glucose units (Taken from Tester et al., 2004b).

The amylopectin molecule is composed of A–, B–, and C–chains (Figure 2.3). A–chains are on the outer surface of the molecule and are attached to the amylopectin molecule by a single linkage and have no branches. Double helices are formed by A–chains which are located within the crystalline lamellae. B–chains are connected to at least two other chains within the amylopectin molecule; their chains are branched and connect A–chains from one side and C–chain on the other side. The branching points within the amylopectin are located in amorphous lamella. The number of A–chains and B–chains are almost the same whereas only a sole C–chain exists in each amylopectin molecule. The C–chain possesses the only reducing end of the amylopectin (Oates, 1997; Buléon et al., 1998). The amylopectin molecule has many non–reducing ends and only a single reducing end. The average length of A– and B–chains in pea starch is 14 to 18 and 45 to 55 glucose molecules, respectively (Ratnayake et al., 2002).

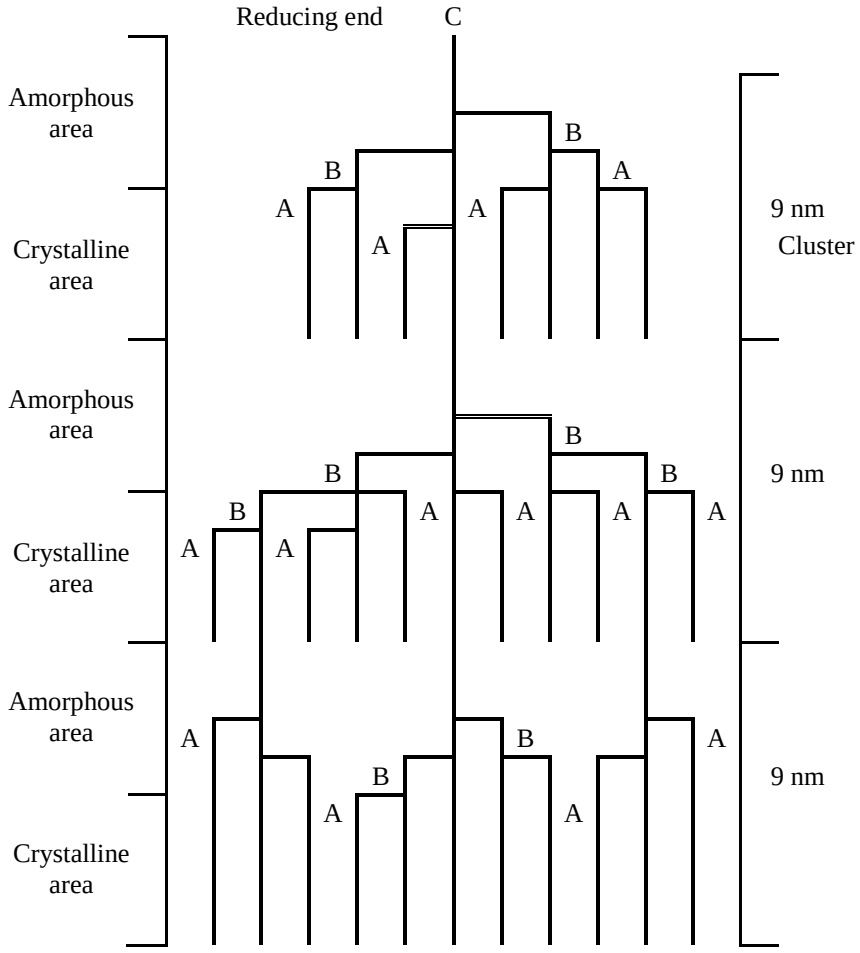

FIGURE 2.3. Schematic model of the molecular structure of amylopectin indicating the branching pattern of the molecule. A–chains are unbranched and linked to B–chains; B–chains are multi–branched chains; C–chain is a sole chain that has the reducing end of the molecule. A– and B–chains contain the nonreducing ends.

2.1.1.3. Lipid and Protein

Lipids and protein may be found in starch granules in association with amylose. Lipids and protein may represent ~1.5% and less than 0.5% of starch granule composition, respectively. Lipids in starch granules are different from surface lipids, which are composed exclusively of lysophospholipids and free fatty acids. In normal starches, the lipid content is proportional to the amylose content; therefore waxy starches

contain a small amount of lipid whereas amylo–starches contain considerably more lipid (Tester et al., 2004a). In pea starch, the amylose–lipid complexes represent between 7.8 to 8.1% of amylose (Ratnayake et al., 2002). Even though the amounts of lipid and protein in starch granules are low, their effect on the physiochemical properties of starch is recognized (Eliasson and Gudmundsson, 2006). Starch structure and function are affected by the amylose–lipid complexes, which might impact starch digestibility. Starch digestion might be reduced as a result of lipid/starch complexes, which reduce the contact between starch molecules and enzyme involved in starch hydrolysis (Svihus et al. 2005). Moreover, as the lipid/starch complexes increase, the degree of starch swelling decreases in response to increasing hydrophobicity. This may reduce starch digestibility, as water is needed for enzymatic degradation. Most of the protein is generated during starch synthesis as biosynthetic enzymes (Jane, 2004). It is associated with lipids on the surface of granules (Tester et al., 2004a).

2.1.1.4. Amylose to Amylopectin Ratio

The proportion of amylose and amylopectin varies among starch sources based on their botanical origin. It has an important impact on starch characteristics and starches can be categorized according to the relative levels of these components. Normal starch from most species contains about 25% amylose and 75% amylopectin. Whereas waxy starch contains less than 15% amylose and often the amount of amylose is negligible, it mainly consists of amylopectin. Starch can be made up only of amylopectin and waxy cultivars of this type are available for many starch sources including barley, maize, potato, rice, sorghum, and wheat. High–amylose (or amylo–) starch is defined as having greater than 36% amylose (Jane, 2004; Tester et al., 2004a). High–amylose variants are

also available with amylose contents up to 70% (Oates, 1997). In general, pulses have a higher content of amylose comparing with other grains. The amylose content of round pea cultivars ranges from 33.1 to 49.6%, whereas wrinkled pea has a range of amylose content from 60.5 to 88% (Ratnayake et al., 2002; Eliasson and Gudmundsson, 2006).

2.1.2. Physical Structure of Starch

2.1.2.1. Starch Granules

Starch is accumulated in granules, which are predominately composed of amylose and amylopectin. However, protein, lipid, mineral, and moisture may also be present in limited amounts in starch granules (Tester et al., 2004a). Starch granules are densely packed and insoluble in water (Oates, 1997). Starch granule size and shape are different among botanical sources (Jane, 2004; Eliasson and Gudmundsson, 2006).

Starch granules range in size from 1 to 100 μm and their shape can be spherical, lenticular, oval, or irregular (Tester et al. 2004b). Examples of starch granule size are barley (2 – 5 μm), wheat (2 – 10 μm), corn (2 – 30 μm), and potato (5 – 100 μm). Moreover, starch granules from barley, corn, and wheat have a spherical shape, whereas potato has rentiform (single) lenticular shapes. The granule size of field pea ranges between 14 – 32 μm (width) and 15 – 37 μm (length) and the shape of starch granules is mostly oval, but round, spherical, elliptical and irregular shapes can be found as well (Ratnayake et al., 2002).

2.1.2.2. Crystalline Structures of Starch

Native starch is a very complex substance and its crystallinity is mainly attributed to the amylopectin structure by means of van der Waal's forces and hydrogen bonds (Imberty et al., 1991; Oates, 1997). Hydrogen bonds are formed between, not within, the

amylopectin chains in double helix formations and between double helices (Imberty et al., 1991). Individual hydrogen bonds are relatively weak, but together they make a strong network. The crystalline structure of starch granules is mostly generated by the double helix formation of glucose chains, which occur within clusters in the amylopectin molecules (Oates, 1997). In most native starches, degree of crystallinity ranges from 15 to 45% (Eliasson and Gudmundsson, 2006). The average crystallinity in smooth pea ranges from 18.9 to 36.5% (Ratnayake et al., 2002).

When starch is viewed using a polarized–light microscope, dark and light zones appear, and this effect is known as birefringence phenomenon. The zones are due to semi–crystalline and amorphous growth rings and are shown in Figure 2.4. The branch points of amylopectin are mainly found in the amorphous area of B–type starches, whereas it is located in both the amorphous and crystalline areas in A–type starches (Jane, 2004). Starch granules are built up in layers starting at the hilum, which is the center of the starch granule and is less organized than the rest of granule (Oates, 1997). The layers of starch granules consist of two alternate regions, semi–crystalline and amorphous. The semi–crystalline region (cluster arrangement) is divided into two areas, a dense area containing parallel chains (crystalline lamella) and a less packed area that possesses the branched points of amylopectin and amylose (amorphous lamella) (Tester et al., 2004a). An amorphous region surrounds the whole cluster region including the amorphous and crystalline lamellae (Imberty et al., 1991). Based on the source of starch, amylose can appear in amorphous or crystalline regions and be found between or among amylopectin clusters (Oates, 1997; Tester et al., 2004a).

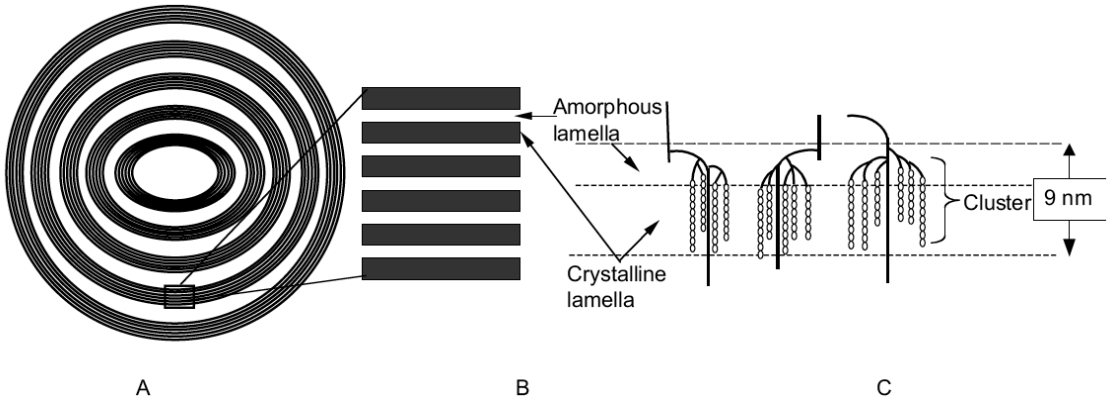

FIGURE 2.4. Schematic diagram represents the starch granule structure. (A) Each granule build–up of concentric rings, alternating semi–crystalline and amorphous. The semi–crystalline growth area contains amorphous and crystalline lamellae areas. (B) The crystalline and amorphous lamellae. (C) The formation of double helix (cluster) structure by adjacent chains (crystalline lamellae) and branching area (amorphous lamellae) (Taken from Tester et al., 2004b).

2.1.2.3. Classification of Starch Based on Structure

Amylopectin from different sources can be classified as A–, B– and C–type starch based on crystallization of starch granules (Buléon et al., 1998). Crystallization of starch granules depends on the length of amylopectin chains and the extent of hydration. When average chains length is between 23 – 29, starch is referred to as A–type (cereal grains and tapioca starches), whereas between 30 – 44 is B–type (root and tubular starches). The C–type is intermediate with chain lengths of 26 – 29 glucose molecules and these starches can be found in bean and pea (Hoover and Sosulki, 1991; Jane et al., 1997; Oates, 1997; Eliasson and Gudmundsson, 2006).

The A– and B–type of starch are the most different forms, whereas C–type contains both A– and B– fractions and is considered an in between form. In general, starches in cereal grains and tapioca are A–type (Jane et al. 1997), tubers such as potato and high amylose (mutant) cereal varieties have B–type starch, and most legumes contain

C–type starch (Gallant et al., 1992; Sajilata et al., 2006; Eliasson and Gudmundsson, 2006). A–type starches are arranged in concentric layers whereas B–type starches are found in eccentric layers when examined microscopically. In general, A–type has an open structure, B–type has a very dense structure and C–type is intermediate. The pea starch is classified as C–type and as noted above contains both A– and B–types of starch (Buléon et al., 1998; Sajilata et al., 2006).

2.2. Starch Digestion

The structure of starch molecules is quite simple compared to other molecules supplying nutrients in animal feed based on the fact that D–glucose is the only monomer linked by only two types of glycosidic bonds; α–1,4 and α–1,6. However, hydrolysis of starch during digestion is a complex process that requires the action of pancreatic and intestinal wall enzymes. The process of starch digestion in chicken has been reviewed on several occasions (Rogel et al., 1987; Moran, 1982, 1985; Gray, 1992; Leeson and Summers, 2001; Carré, 2004; Pesti et al., 2005) and these reviews will be cited in the following paragraphs.

According to Moran (1985) and Leeson and Summers (2001), chickens are adapted to starch digestion at a very early age. For example, α–amylase, maltase, and isomaltase reach their maximum production within four days after hatching. Also, active transport of glucose is found two days after hatching and its activity increases during the first 4 weeks of life. Moreover, pancreatic α–amylase secretion is positively related to the amount of ingested starch (Moran, 1985).

The native starch granules resist digestion so it is important to disrupt their structure physically in order to facilitate chemical hydrolysis by amylases. Even though

some amylase might be found in saliva and crop of chickens, a significant starch digestion prior to the proventriculus has not been confirmed (Leeson and Summers, 2001; Pesti et al., 2005). Starch digestion in chicken can be divided into three steps: initial soaking in the crop, physical disruption of feed by the action of grinding in the gizzard, and finally the chemical hydrolysis by pancreatic and brush–boarder membrane enzymes.

Ingested feed passes to the crop and proventriculus where the wetting action of saliva and water occurs. This speeds up the digestion of starch, however the swelling of starch granules may be limited because of variable holding time in the crop (Leeson and Summers, 2001). Afterward feed passes to the gizzard where physical disruption by grinding takes place. In general, starch digestion in poultry occurs in the small intestine by the action of pancreatic and brush–border membrane enzymes. Starch hydrolysis by pancreatic α–amylase is the most limiting factor and brush–border membrane enzymes have the complementary action. Starch digestion is initiated by pancreatic enzymes attacking the outer surface of starch granules, as starch granules are water–insoluble. In fact, only small portions of the surface of starch granules are susceptible to pancreatic enzymes (Rogel et al., 1987).

In the lumen of the small intestine, α–amylase is secreted from the pancreas and it is the only carbohydrase dissolved in the luminal fluid. It has an optimal pH of 6.9 and it only hydrolyses the α–1,4 bonds with some restriction at branching points (Rogel et al., 1987). The starch degradation by α–amylase is incomplete; amylose is hydrolyzed to maltose (disaccharide) and maltotriose (trisaccharide) and amylopectin is broken down to maltose, maltotriose, and small–branched α–dextrins (Gray, 1992). Starch digestion starts in the duodenum and continues through the jejunum and ileum; however, it is believed

that most starch digestion by pancreatic amylase takes place in the jejunum because chickens have an ample amount of α–amylase for starch digestion (Rogel et al., 1987).

The action of α–amylase begins with a random attaching of its catalytic sites to five glucose molecules adjacent to the reducing end of starch. The enzyme attaches only to α–1,4 glycosidic bonds and has no specificity for the α–1,6 linkage in amylopectin. After the attachment, the linkage between the second and third of glucose residues is cleaved and then the enzyme slides over the glucose molecules towards the non–reducing end. In each cleavage, maltose is released and at the end of chain, maltotriose remains. In addition, α–amylase has less specificity to glycosyl oligosaccharides with two or three glucose molecules. The capacity of α–amylase to hydrolysis α–1,4 linkages adjacent to the α–1,6 linkages is strictly prevented. As a result, α–dextrins with one or more α–1,6 linkages are produced from amylopectin hydrolysis (Moran, 1982; Gray, 1992).

Only free glucose is absorbed through the intestinal wall therefore maltose, maltotriose and α–dextrins must be hydrolyzed into free glucose. They are broken down by oligosaccharidase glycoproteins that are found in the intestinal surface of the brush border membrane. These enzymes include amyloglucosidase, sucrase, and α–dextrinase (isomaltase). Amyloglucosidase is able to remove single glucose residues one after the other from the non–reducing end of the small α–1,4 chain but like α–amylase it has no ability to hydrolyze α–1,6 linkages. Sucrase and dextrinase are initially synthesized as one glycoprotein chain (sucrase–α–dextrinase) in the enterocyte. This hybrid carbohydrase is cleaved by pancreatic proteases into sucrase and α–dextrinase after its insertion into the brush border membrane. Sucrase hydrolyzes α–1,4 glycosidic bonds and it complements the specificity of amyloglucosidase by preferring shorter chains like

maltose and maltotriose. The non–reducing end of α–1,6 bond is cleaved by isomaltase (α–dextrinase). The final products of starch hydrolysis by α–amylase, amyloglucosidase, sucrase, and α–dextrinase are glucose molecules, the monosaccharide that is transported across the intestinal membrane.

Glucose is mainly absorbed into the bloodstream by an active transporter. A specific glycoprotein carrier expressed in the brush border of the gut wall transports glucose molecules through the small intestine membrane. It has a high affinity for monosaccharides and it is driven by Na–K–pump. In order to facilitate glucose absorption, the presence of Na^+ in the luminal glucose solution is needed. Glucose is transported against gradient into the enterocyte by binding to two ions of Na^+ from one side and the transporter on other side. Both glucose molecule and Na^+ ions are carried and released in the enterocytes. The intracellular Na^+ ions across the basolateral membrane are pumped back by Na–K–pump and glucose is transported to the capillaries by diffusion. However, glucose can also be transported by another protein carrier at the basolateral membrane (Gray, 1992).

In the gut wall, some of the absorbed glucose will be utilized (oxidized) as a source of energy by the gut itself. Around 30% of absorbed glucose is converted to lactate in the small intestine (Pesti et al., 2005). The other portion will be taken up via the portal vein to the bloodstream, and provide peripheral tissues with energy or be stored as glycogen (muscles and liver) or converted to fat for future energy needs. In general glucose will be utilized in the animal metabolism. The glucose uptake by cells and glucose homeostasis are controlled by the action of insulin and other hormones. The

excretion of insulin is stimulated by increasing of blood glucose and it stimulates the synthesis and storage of glycogen in liver and muscles.

A variable fraction of resistant and undigested starch escapes absorption and enters the hindgut. Only a limited part is fermented by the micro–flora residing in the hindgut. The end product of starch digestion in the small intestine is glucose whereas volatile fatty acids (VFA), methane, hydrogen, and carbon dioxide are the products of fermentation by the micro–flora in the hindgut. The energy value of the end products of starch fermentation is less efficient as they cannot be utilized in poultry metabolism. Therefore, the energy value of indigestible starch is lost in the form of fermentation heat and other products.

In summary, starch is enzymatically hydrolyzed in the small intestine to glucose and then it is absorbed. First, starch is broken down into maltose, maltotriose, and α–limit dextrin by pancreatic α–amylase. Afterward, these oligosaccharides are hydrolyzed into glucose by the complimentary action of brush border enzymes in the small intestine wall. The final product of starch digestion is glucose, which is absorbed from the small intestine into the bloodstream.

2.2.1. Factors Affecting Starch Digestion in Poultry

Starch is the main energy source in poultry diets. Therefore, factors affecting its digestibility will impact energy availability. Starch is considered readily digested in poultry, but starch from grain and pulse seeds was found not to be completely digested in broiler chickens (Longstaff and McNab, 1987; Yutste et al., 1991; Svihus and Hetland, 2001; Weurding et al., 2001b). The rate of starch digestion through the gut and the extent of starch digestion at the end of the ileum are mainly affected by starch characteristics,

feed processing, and gastrointestinal tract conditions (Classen, 1996; Wiseman et al., 2000; Tester et al., 2004b; Carré, 2004; Enting et al., 2005).

The susceptibility of starch granules to the action of digestive enzymes is the main factor that affects starch digestibility. Starch susceptibility is determined by different starch characteristics such as amylose to amylopectin ratio, degree of crystallinity, size of the starch granules (surface area), association with matrixes of lipids, protein, and polysaccharides, and encapsulation of starch granules in the cell walls, as well as the physiochemical (e.g. viscosity) nature of the gut digesta (Moran, 1982).

The degree of accessibility of starch for enzymatic hydrolysis is mainly affected by the physicochemical structure of the seeds. The size of starch granules varies based on their botanical origin, cultivar, and environmental growing conditions. Moreover, granule size affects starch digestibility (Svihus et al., 2005; Singh et al., 2010). The ratio between surface area and granules volume determines the potential hydrolysis of starch by digestive enzymes. The larger granules size, the smaller the proportional surface area and vice versa. As the granule size decreases, surface area increases, and starch digestion improves (Franco et al., 1992; Tester et al., 2004b). Moreover, the smaller the granules size, the less crystalline structure, the higher the starch digestion (Buléon et al., 1998; Svihus et al., 2005).

As the proportion of crystallinity increases in a starch granule, the degree and rate of starch digestion decreases. The open structure of A–type starch facilitates the action of digestive enzymes; as a result starch will be digested rapidly. In contrast, B–type starch resists the action of digestive enzymes and therefore is digested slowly because of its

dense structure. It can be concluded that starch with A–type is the most digestible whereas B–type is the less digested and C–type is the intermediate (Oates, 1997).

Another starch structure factor that related to starch digestion is amylose to amylopectin ratio. The ratio of these two polymers has an effect on the digestibility of starch (Carré, 2004; Svihus et al., 2005). The difference between amylose and amylopectin digestibility is mostly related to their structures. The surface area of amylopectin is substantially larger than amylose and therefore amylopectin is more susceptible to amylase action compared with amylose. Furthermore, the chain length in amylopectin is much shorter compared with amylose so more hydrogen bonds link amylose chains than in amylopectin; as a result amylose is less susceptible to digestive enzymes attack compared with amylopectin (Zoble, 1988). As starch amylose content increases (> 40%), the B–type of crystalline structure is formed and starch digestibility decreases (Carré, 2004). However that is not always the case as observed with high amylose barley (45%), which has the same digestibility as the normal starch cultivars (Bergh et al., 1999). On the other hand, waxy starches (low amylose content) such as found in sorghum have a higher starch digestibility (Roony and Pflugfelder, 1986). Legume grains such as pea are characterized by a high content of amylose (Hoover and Sosulski., 1991), which in turn impacts on their digestibility (Weurding et al., 2001b).

Lipid and protein in starch granules may have a negative impact on starch digestibility (Classen, 1996; Svihus et al., 2005). Their action is by reducing swelling of starch granules and by reducing the contact of starch granules with the digestive enzymes. Most lipids are found on the cell wall of starch granules (Baldwin et al., 1997). Lipids can complex with starch and reduce starch digestion by increasing the

hydrophobic nature of starch granules and preventing access by digestive enzymes (Vasanthan and Bhatty, 1996). Crowe et al. (2000) reported that complexes are formed between fatty acids and amylase, which may reduce amylose digestion. In contrast, it has been reported that a firm complex cannot be formed with amylopectin due to it is structure (Zoble, 1988). Lipid or protein encapsulation may also reduce swelling of starch granule during feed processing, milling and gelatinization, further reducing the potential digestibility of starch.

Starch and protein are found within the cell wall matrix and the complex nature of cell wall structure makes starch and protein less accessible for digestive enzymes of poultry (Longstaff and McNab, 1987). Starch granules are embedded in protein matrix and the accessibility of enzymes to starch granules depends on how rapidly the protein matrix is digested. Starch granules can be encapsulated or be closely associated with protein that impact starch digestion. This has been clearly demonstrated in ruminant species (McAllister et al., 1993). This layer of protein may reduce the accessibility of digestive enzymes to starch granules (Classen, 1996); however protein digestion usually takes place before starch digestion (Duke, 2002; Pesti et al., 2005). Therefore, protein that encapsulates starch should be digested prior to starch hydrolysis by digestive enzymes.

Starch in cereal and pulse grains is accumulated within the seed endosperm, which contains starch granules enclosed in cell walls. In poultry feed ingredients, the cell wall contains substantial amounts of non–starch polysaccharides, for example, arabinoxylans appear in wheat and β–glucans found in barley. The cell walls can act as barriers to digestive enzymes, which may reduce utilization of starch. Also, released

soluble non–starch polysaccharides (NSP) may reduce digestion of starch and other nutrients by increasing digesta viscosity that alter the digestion process and can also affect gut micro–flora (Classen, 1996; Refstie et al., 1999).

Naturally occurring anti nutritional factors (**ANFs**) can reduce nutrient digestibility by inhibiting digestive enzymes (Leeson and Summers, 2001; Carré, 2004; Pesti et al., 2005). However, cereal and legume cultivars are selected for high nutrient digestibility, and as a consequence have low levels of ANFs. Starch digestibility is reduced by the presence of ANFs such as amylase inhibitors, condensed tannins, and water–soluble NSP. Animal feed is often processed in order to inactivate these ANFs. Wheat, sorghum, rye, and triticale have high levels of α–amylase inhibitors, whereas they have not been found in barley, maize, and rice. Fortunately, α–amylase inhibitors have high sensitivity to pepsin hydrolysis in chickens (Rogel et al., 1987) and this may partially account for the finding that α–amylase inhibitors have negligible effects on starch digestibility in chickens (Carré, 2004). Chickens are able to increase pancreatic α–amylase as α–amylase inhibitors increased. Condensed tannins are located within seed hulls in some pea, faba bean, and sorghum cultivars. However, starch digestibility is only reduced by very high levels of dietary condensed tannins (Longstaff and McNab, 1991).

2.2.2. The Kinetics of Starch Digestion

In the small intestine, starch digestion and more specifically the rate and the extent of starch digestion are affected by a number of factors. The physical and chemical characteristics of starch, conditions of gastrointestinal tract, and feed formulation and processing are the most important factors that determine starch susceptibility to digestive enzymes (Carré, 2004; Tester, 2004b; Wiseman, 2006; Svihus et al., 2005; Singh et al.,

2010). In fact, these factors vary among feedstuffs because of inherent differences and growing conditions (Yutste et al., 1991; Weurding et al., 2001b). Starch utilization is most efficient when starch is digested in the small intestine because starch is broken down into glucose and absorbed by the intestine wall. The degree and efficacy of starch fermentation post–ileum in the chicken is low at best.

Although an apparent ample amount of amylase in the small intestine of chickens, the rate of starch digestion and glucose absorption are affected by multiple factors. The kinetics of starch digestion is affected by its structure. When the crystalline arrangement of starch is highly packed, A–type, starch would be slowly digested. In contrast, the amorphous structure of B–type starch is rapidly digested (Sajilata et al., 2006). Branched and long chains in starch granules result in SDS and therefore affect the kinetics of starch digestion. Depending on the conditions of feed processing and the source of starch, the kinetics of starch digestion will be affected and the rate of digestion slowed (Singh et al., 2010).

Feed digestibility can be affected by the passage rate of the diet through the gastro–intestinal tract (**GIT**). Two factors that regulate the passage rate of feed through the GIT are the density and the bulk of digesta (Duke, 2002). For example, complex carbohydrates, which are not easily digested, pass more slowly than easily digested molecules.

In the small intestine of broiler chickens, the extent of starch digestion determines the amount of energy that is provided by dietary starch. In fact, the extent of starch digestion is positively correlated to the AME content of the diet (Wiseman, 2006). On the other hand, the differences of the site and rate of starch digestion may have metabolic

consequences that affect feed utilization and chickens performance. A slowly digested starch might have the same extent of starch digestion as the rapidly digested starch, but have different amounts of starch digested at specific sites of the small intestine (Figure 2.5). In other words, a rapidly digested starch may be completely digested at the end of jejunum whereas a slowly digested starch will be digested completely in the ileum.

The rate of starch digestion may have benefits in poultry nutrition as it may affect the rate of glucose absorption and availability throughout the small intestine. The exact location of starch digestion and absorption may relate to synchronization of energy and protein absorption and subsequent post absorption metabolism. Synchronization of available energy from glucose with amino acid digestion and absorption could increase the efficiency of protein deposition and as a result improve animal performance (Weurding et al., 2003). Differences in starch digestion site may also have metabolic consequences that affect nutrient utilization. Glucose absorption rate affects insulin response, which in turn can affect protein accretion. The site of starch digestion might also determine where it is utilized, in splanchnic tissue or post–absorption. Research has suggested that including a SDS in broiler diets improves broiler performance and if confirmed, this would suggest that both the rate and extent of starch digestibility should be considered in feed formulation.

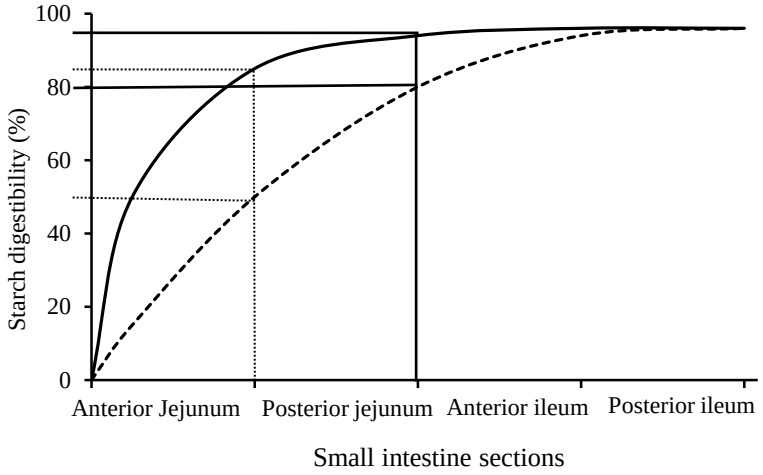

FIGURE 2.5. Two starch sources with different rates but the same extent of starch digestion. The solid line represents RDS and the dashed line represents SDS.

2.2.3. Nutritional Classification of Starch

Based on the timeline of digestion in the small intestine, starch can be classified as rapidly digested, slowly digested, and undigested (resistant) (Englyst et al., 1992, 1999).

2.2.3.1. Rapidly Digested Starch (RDS)

The hydrolysis of this starch type is complete by the anterior part of the small intestine. Leeson and Summers (2001) reported that most of starch digestion occurs in the jejunum.

2.2.3.2. Slowly Digested Starch (SDS)

Slowly digested starch is completely digested by the end of ileum but the rate of digestion in the small intestine is slow. This type of starch is usually physically protected from the action of pancreatic amylases. The intact structure of cereal or pulse grains and the rigidity of cell walls impact the amount of SDS. Feed processing has been reported to

reduce physical and other barriers to starch digestion and thereby influence the amount of SDS (Oates, 1997).

2.2.3.3. Resistant Starch (RS)

This portion of starch resists hydrolysis by pancreatic amylases in the small intestine and escapes into the hindgut (Oates, 1997). The term resistant starch was first introduced in human nutrition (Englyst et al., 1982). It is defined as "the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals" EURESTA (European Resistant Starch Research Group). The amount of RS depends on the chemical and physical characteristics of starch and feedstuffs, feed processing, gastrointestinal tract conditions, absorption capacity, feed transit time, and the activity of digestive enzymes.

Englyst et al. (1992) classified RS into three different portions; physically resistant starch (RS1): starch that is physically inaccessible to enzymatic attack due to proteins and intact cell walls encapsulating starch granules; granule resistant starch (RS2) where the structure of the starch granules prevents digestion; and retrograded resistant starch (RS3), which results from retrogradation of gelatinized starch.

2.2.4. Glycemic Index (GI)

In human nutrition, the concept of glycemic index (**GI**) was first introduced by David Jenkins and co–workers in 1981 at the University of Toronto. It reflects the effect of glucose absorption rate on blood glucose level and therefore ranks food based on how much the level of blood glucose is raised (Figure 2.6). Using the postprandial blood glucose response, carbohydrate–based food can be categorized into high, medium, and low GI (Jenkins et al., 2002). The GI is defined as "the incremental blood glucose area

following the test food, expressed as the percentage of the corresponding area following a carbohydrate equivalent load of a reference product" (Björck et al., 2000). The classification of dietary carbohydrate based on their GI has been used to highlight the potential benefits of eating a diet with low GI.

The GI value is determined by comparing the blood glucose level after consuming a test available carbohydrate (50g) to the same amount of available carbohydrate from the reference food (Jenkins et al., 1981). The reference dietary carbohydrate is either a pure glucose solution or white bread. The blood glucose curve of test carbohydrate is expressed as percentage of that under the reference carbohydrate. GI value is mainly affected the rate of starch digestion and glucose absorption. Based on dietary GI, foods are ranked with high GI that dietary carbohydrate is digested rapidly and raises the blood glucose level whereas low dietary carbohydrate is digested slowly and related to the low blood glucose level.

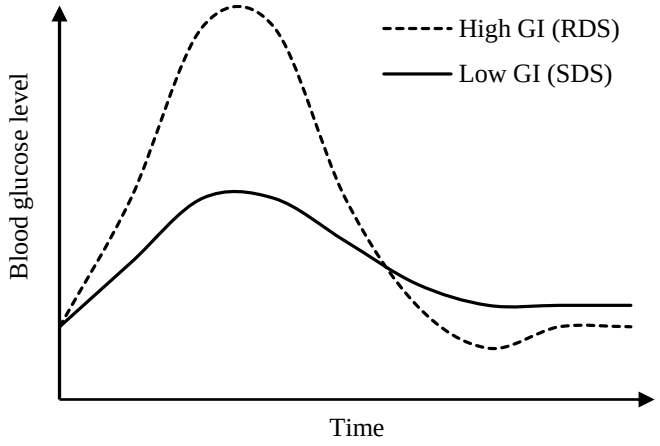

FIGURE 2.6. Effects of RDS and SDS on postprandial glucose level over time after a meal.

Consuming a diet with a low GI will reduce the level of blood insulin (Jenkins et al., 1982, 2002) as it is well correlated to the glycemic effect. Absorbed glucose stimulates β–cells to release insulin and the level of insulin released is related to the extent and the rate of absorbed carbohydrate. Hence, consuming a diet with a low GI is a dietary tool to modulate the rate of glucose absorption and insulin response. In addition, it prolongs glucose absorption, which may have metabolic advantages. In human nutrition, consuming slowly absorbed carbohydrate (low GI) has been suggested to provide metabolic benefits in regard to risk of coronary heart disease, diabetes, and cardiovascular disease. Moreover, it lowers the level of blood glucose and insulin and improves blood lipids (Jenkins et al., 2002). Foods with low GI are recommended for athletes to prolong their physical endurance and for obese people to prolong satiety and reduce food intake (Björck, 2006).

The GI value is affected by all the factors that affect starch degradation and glucose absorption (discussed in factors affecting starch digestion). The rate of starch degradation in the small intestine and the speed of food transit in the gut determine the glucose absorption rate, in fact, it determines both the duration and extent of blood glucose after a meal. Slowly digestible starch that has a medium to low GI and thus reduces the glycemic load of a food product compared to rapidly digestible starch with a high GI. Moreover, GI is well correlated with the rate of starch digestion (Englyst et al., 1999). Jenkins et al. (2002) have indicated a good relationship between the rate of in vitro degradation and the glycemic response. It can be concluded that the rate rather than extent of starch digestion is rate–limiting step for blood glucose level.

2.3. Consequences of Starch Digestion Rate in Poultry

The kinetics of starch digestion may have important nutritional and metabolic consequences. The corollaries of feeding RDS or SDS on animal metabolism can be predicted. Rapid or slow starch digestion may elicit different metabolic and hormonal responses in animal metabolism. However, the information regarding the kinetics of starch digestion and poultry performance is minimal in comparison to research for mammalian species. There are good reasons to believe that SDS may offer a range of nutritional benefits due to its stabilizing and sustaining effects on blood glucose level, as well as providing a better glucose supply to the posterior part of the small intestine (Weurding et al. 2003a). It can be suggested that SDS slows and moderates the increase of postprandial blood glucose levels, and sustains and prolongs the increased blood levels over time after feeding. In contrast, RDS will result in a fast and high peak of postprandial blood glucose levels followed by a rapid decline, partially under baseline after feeding (Figure 2.6). Moreover, SDS results in different metabolic and hormonal responses compared with RDS. These responses may have an impact on the GI of feed, which may affect animal performance and satiety (Jenkins et al., 2002; Weurding et al., 2003b; Lehmann and Robin, 2007).

Protein synthesis and degradation are affected by insulin level, which in turn affect the growth rate of animals. Maintaining optimum insulin levels may promote more active and efficient muscle deposition. A moderate and prolonged glucose supply from SDS will lead to a lower but longer insulin curve. In contrast, RDS would result in a higher and shorter insulin curve (Björck et al., 2000).

It could be assumed that SDS is digested throughout the small intestine, not only in anterior sections. This results in an increased flow and supply of glucose into the posterior part of the small intestine, which provides enterocytes with more available glucose. Glucose can be metabolized instead of amino acids as energy source and therefore spare the use of amino acids for this purpose by the lower part of the small intestine. Consequently, this improves energy and protein utilization, as it makes amino acids available for muscle growth (Gray, 1992).

When the amount of available glucose at the posterior part of the small intestine is limited, enterocytes utilize other nutrients such as AA as a source of energy. It results in more AA being catabolized for energy in the gut and less AA available for protein synthesis. In summary, feeding SDS synchronizes energy and protein metabolism, provides enterocytes of the posterior part of the small intestine with energy, as well as maintains available energy for AA absorption and metabolism, which may result in improved animal performance.

The relation between slow rate of starch degradation and glucose absorption from pea has been examined in human nutrition since it modulates the peak in post–meal insulin production (Jenkins et al., 1981). Moreover, high amylose/amylopectin ratio pea genotypes have corresponding reduced glycemic effect. The corollary of slow digested starch was also investigated in poultry nutrition. An experiment was conducted using broiler chickens to determine the effect of slow and rapid digested starch on broiler performance. Rapid digested starch was defined as being degraded rapidly with absorption in the jejunum, whereas slow digested starch was defined as being degraded slowly with glucose absorption in the ileum. Examples of rapid degraded starch sources

are corn, tapioca, and rice whereas pea is a source of slow digested starch (Weurding et al. 2001a). It was reported that including a minimum quantity of slow degraded starch (pea starch) in a broiler diet improved growth rate and FCR compared to an iso–caloric diet containing only rapid digested starch. Broilers fed diets containing slow digested starch had improved amino acid utilization as shown by their response to increased levels of dietary lysine (Weurding et al. 2003b). The effect of feeding slow digested starch from pea was explained based on corollaries, which are related to continuous supply of glucose; stimulating prolonged insulin production, which has effect on protein accretion; providing a direct source of energy for posterior sections of the gut; sparing the catabolism of gluconeogenic amino acids; improving the energetically efficient of glucose by minimizing conversions between glucose and its storage molecules, which are formed because glucose spikes following a meal of rapid digested starch.

While, there is substantial evidence supporting the advantages of consuming a low GI food in human nutrition, most conventional feedstuffs included in poultry diets are high GI. However, Weurding et al. (2003a) reported that there are differences in the rate of starch digestion between feedstuffs. Pea starch is digested slower than tapioca starch. Pea starch is higher in amylose and predominated by C–type of starch, and amylose is less digested by non–ruminant animals than amylopectin. Also C–type of starch molecules is more resistant to digestive enzymes than A–type. It could be suggested that feeding pea in poultry diets may improve poultry performance. However, as mentioned previously, it is a challenge to predict the benefits of feeding a low GI diet due to the complexity of all factors and their interaction.

2.4. Feed Processing

Feed processing typically refers to particle size reduction and the application of an array of hydrothermal conditions. It is applied to feedstuffs and formulated diets in order to improve nutritional value and improve poultry performance (Behnke, 1996; Pesti et al., 2005). In feed mills, processing can include grinding, rolling, mixing, crumbling, roasting, micronizing, extruding, expanding, and cold and steam pelleting. However, the two classical feed processes commonly used in the poultry feed industry are grinding and pelleting.

In the poultry feed industry, grinding and pelleting are often combined, which adds complexity to evaluating their efficacy. Moreover, understanding the impact of feed processing is difficult because of the variable combinations of feed ingredients used in poultry diets and the different responses of feed ingredients to the processing applied. As starch is the major energy–yielding component of poultry feedstuffs, the effect of feed processing on starch structure and digestibility is required to accurately formulate diets. Feed processing can alter the structure of starch granules, increase their susceptibility to digestive enzymes and thereby increase starch digestibility.

2.4.1. Grinding

As chickens age, they gain the ability to grind and utilize (at least to some degree) whole grains as a result of the grinding action of their gizzard (Svihus, 2011). However, grinding feed ingredients is generally applied in conventional processing. The most common grinding technique is hammer milling, a process that reduces particle size and thereby increases the surface area for digestion. Cereal and pulse grains are also ground prior to mixing in order to get a homogenous mixture (Pesti et al., 2005), decrease

segregation of feed ingredients, and facilitate feed pelleting (Behnke, 1996). The main variable in grinding is the screen–hole size, which ranges between 2.0 to 8.0 mm in poultry feed mills.

The use of a specific screen size is relates to the eventual feed form (mash or pellets), cost of processing and the convenience of mixing the raw materials. In the case of mash feeding, fine grinding can reduce feed intake and indeed, extreme fine particle sizes may cause beak necrosis. This is particularly true for wheat where fine grind results in feed that adheres to bird beaks as a result of wheat gluten content.

2.4.2. Pelleting

Pelleting is the process of making homogenous pellets from mash feed. Typically mash diets (with cylindrical feed particles commonly between 2.5 and 5.0 mm in diameter) are treated by the addition of steam in a conditioning chamber to increase moisture content and temperature (82 to 90°C). After conditioning, pellets are formed using physical compaction to push feed through dies of various sizes (range for poultry 2.5 – 5.0 mm). The moisture content of the pellets after it leaves the die is between is 15 to 18% (Pesti et al., 2005) and as a consequence pellets are dried to improve pellet quality and prevent undesirable microorganism growth. Young chicks are not able to consume pellets with a size of > 2.5 mm and therefore pellet size is reduced by passing them between two grooved rollers to produce crumbles (Pesti et al., 2005).

Pelleting is used in poultry feed for a number of reasons (Behnke, 1996; Pesti et al., 2005). These include to reduce wastage and feed dustiness, minimize feed selection, decrease segregation of feed ingredients, reduce the energy spent for prehension, destroy pathogenic organisms (e.g. salmonellae), enhance feed texture, improve palatability and

feed consumption, increase the bulk density of feed (nutrient density), facilitate feed transportation, and expand feed ingredient handling which allows the use of alternative feed ingredients in order to reduce the feed cost. Moreover, pelleting may improve nutrient digestibility by gelatinizing starch and denaturizing the heat labile ANFs.

Commercial pelleting most often involves steam conditioning but the process can also occur without this step. This is termed as cold pelleting. Temperature exposure in cold pellets due to the friction of the process is between 37 – 65°C depending upon the feed ingredients used in diet. Moreover, pelleting might neutralize the effect of grinding. For example, if the diets are pelleted, fine grinding is not needed to improve legume starch digestibility (Conan et al., 1992).

Research to determine the digestibility of feed ingredients and also in some production experiments fail to provide a complete description of all aspects of feed processing, and as a consequence the value of the results are reduced. The fact that the optimum processing conditions vary among feed ingredients increased the need for a complete understanding of diet processing in nutritional research.

2.4.3. Gelatinization

Native starch is insoluble in water because of the semi–crystalline nature of its granules and the presence of hydrogen bonds between helices. However, when it is heated above the gelatinization temperature in the presence of sufficient water, starch undergoes irreversible changes, and becomes soluble in water. This process is known as gelatinization and the change in solubility is due to the loss of crystallinity of starch granules. In gelatinized starch, the crystalline structure of amylopectin is disrupted and the cluster chains are randomly arranged. Therefore, starch granules are swollen and the

cell wall is ruptured (Jane, 2004; Zobel, 1988, 2006). In summary, gelatinization is a transition from order to disorder of starch molecules

The first step during this process is swelling of starch granules, which is caused by water uptake. The water is taken up first by the amorphous regions and subsequently by the crystalline regions at a slow rate. Water penetration into the amorphous regions causes swelling, and provides force to break hydrogen bonds in the crystalline regions; as a result starch starts to swell and lose its birefringence. Granules become bigger, and no space is left between granules, which results in a firm substance. With a further gelatinization, the molecular structure of starch is disrupted as intermolecular hydrogen bonds are broken. In general, the starch structure is transformed from semi–crystalline to amorphous regions. During the gelatinization process some of amylose molecules are released and the free polymers are dissolved, which in turn increases viscosity. The temperature needed for gelatinization is dependent on the amount of available water (Rooney and Pflugfelder, 1986).

As noted previously, gelatinization is a result of a combination of heat and moisture and the temperature required for this process is dependent on the starch source. The temperature when 5% of starch granules start to gelatinize is defined as T0, while the higher temperature required to gelatinize 95% of starch granules is termed Te. T0 and Te represent the temperatures when 5 and 95% of starch has lost its birefringence, respectively (Sablani, 2008). The ranges and beginning (T0) and final (Te) gelatinization temperatures of starch sources are affected by the water content of starch.

Gelatinization is influenced by the physiochemical structure of starch including amylose and amylopectin characteristics, chain lengths, degree of branching,

amylose/amylopectin ratio, and amylose-lipid complexes. As a result, the gelatinization temperatures of feed ingredients are variable. Gelatinization in cereal grains and pulses is initiated within the range of 60 to 70 °C. The gelatinization temperature range of barley starch is relatively low and wide, whereas pea has a higher gelatinization temperature with a narrow range.

Gelatinization can be induced mechanically and/or thermally; however, the presence of free water is the most critical condition (Zobel, 1984). When water is limited, a higher temperature is needed to gelatinize the amorphous area. During milling and grinding of feedstuffs, mechanical gelatinization of starch may occur, however the amount of gelatinized starch is small and dependent on starch origin and moisture content. In poultry feed, gelatinization may occur during the pelleting process. However most feed processing (including pelleting) occurs under relatively dry conditions, which in turn means that a high temperature (> 120°C) is needed to gelatinize starch (Carré, 2004). In the case of pelleting processes, only the outer surface of pellet may reach the high temperatures required to gelatinize starch. Therefore only a small part of starch will be retrograded (discussed later) so the impact of pelleting on starch digestibility may be negligible (Carré, 2004; Eliasson and Gudmundsson, 2006; Sablani, 2008; Zoble and Stephen, 2006).

2.4.4. Retrogradation

Gelatinization is an irreversible process, but in some circumstances starch may re-crystallize after cooling and this process is termed retrogradation. In general starch retrogradation occurs after gelatinization, but the process is not the exact reverse of gelatinization (Jane, 2004). When starch is cooled after gelatinization, the water is pushed

out and the free polymers form a rigid network (gelation) that no longer resembles native starch. In retrograded starch, the crystallinity is primarily caused by amylose and to a lesser degree by amylopectin. Amylose chains are linked together in an ordered structure that resembles the B–type pattern of starch and the length of chains in the amylose molecules affects the degree of retrograded starch. Starch with a higher proportion of amylopectin tends to be more slowly retrograded than starch that has higher amylose content. Amylose can be retrograded within a short period of time (minutes to several hours), whereas amylopectin may take up weeks to be retrograded. As starch sources vary in the length of side chains in amylopectin (A–, B–, and C–type); the rate of starch retrogradation is affected by type of starch (Hoover, 1995).

Gelatinization and retrogradation of starch are processes that occur when starch is heated in the presence of excess water. In the feed industry, retrogradation is an undesirable change in starch structure as it becomes more resistant to the hydrolysis by digestive enzymes. However, in the feed industry, little water is added to feed processing so it is generally believed that retrogradation is limited and of little importance in pelleting feed.

2.4.4.1. Effects of Feed Processing on Energy Value of Pea

Feed processing impacts nutritional value of formulated diets through changes in nutrient digestibility and energy availability. Carré et al. (1987) reported no effect of pelleting on AME_n values of wheat– and corn–based diets, but in contrast, AME_n values of pea–corn or pea–wheat based diets were improved by pelleting. The improvement in AME_n was explained as a result of improvements in starch and protein digestibility. Longstaff and McNab (1987) using adult cockerels, found that the TME_n of whole pea seeds is significantly lower than ground pea seeds (1–mm sieve size), 2368 vs. 2719 kcal/kg; respectively. However, none of the other feed processing techniques (heating, autoclaving, and dehulling) examined was able to improve the energy value of pea seeds. Carré et al. (1991) investigated the effect of feed form on the energy value of pea. Pea seeds were ground using 2.0–mm screen–hole size. Mash or pelleted diets were fed to adult cockerels and 25–day–old broilers. The energy value of pea was increased by pelleting using a 4 × 30 mm die with flow rate 2.47 kg/min and inlet temperature of 75°C and an outlet temperature of 76°C. The improvement in AME_n values (3,066 vs. 2,811 kcal/kg DM) for adult broilers and (3,014 vs. 2,761 kcal/kg DM) for young broilers fed pelleted and mash pea; respectively. They also concluded that there were no significant effects of bird age on the value of AME_n. Brenes et al. (1993) reported that the mash form of pea–based diet had lower AME_n value in 14–days–old broilers compared to the same diet after autoclaving and dehulling; however, the nature of the effect of processing was also related to pea cultivar. Chicken performance (17–d–old) was improved by pelleting (80ºC) and expansion compared to ground canola–pea based diets (1.5–mm screen size). The diet AME_n values were 2877, 3087, and 3028 kcal/kg for untreated

(ground), pelleted, and expanded diets; respectively (Fasina et al., 1997). Grosjean et al. (1999) determined the AME of pea fed to ISA Brown cockerels. After grinding using a 3.0–mm screen–hole size and feeding in mash or cold–pelleted form, the average AME values were 2851 and 3150 kcal/kg (DM); respectively.

As nutrient digestibility is improved by processing, the ME of feed is enhanced. However, the optimum grind size, pelleting–conditioning temperature, and other processing applications can vary with feedstuff. Therefore it is very complicated to recommend the same feed processing for all formulated diets. The inconsistent data reported about energy value of pea for poultry may result in increasing the safety margin when pea is included in poultry diets. The energy of pea–based diets is affected by bird's age and it is better utilized by adult birds compared to young chicks. However, in most studies the diet processing including grinding and pelleting conditions are missing. Moreover, in some studies ME was reported on AS IS basis others on DM basis yet in few literatures it is missed. Therefore the value of AME that used in feed formulation should be coordinated with processing conditions that applied for poultry diets.

2.4.4.2. Effects of Feed Processing on Starch Digestibility of Pea

Processing has a major impact on starch digestibility as a result of reducing starch granule size, and disrupting the crystalline structure of starch and subsequent formation of amorphous structure (Tester et al., 2004b; Pesti et al., 2005). Starch digestibility of pea was improved by grinding using 1 mm sieve size in adult cockerels compared to whole pea, 88.1 vs. 75.6%; respectively. The improvement of starch digestibility of pea seeds was caused by an increase in the accessibility of starch granules to amylase action (Longstaff and McNab, 1987). Starch digestibility of corn–pea and wheat–pea based diets

was improved by steam pelleting (Carré et al., 1987). Also steam pelleting had improved starch digestibility of spring–pea cultivars for both young and adult cockerels. It also reduced the variability of starch digestion between pea cultivars (Carré et al., 1991). In poultry, reducing the mean particle size of seed flours from 1000 to 300 µm has improved starch digestibility of faba beans (Totsuka et al., 1977; Lacassagne et al., 1991) and pea (Conan et al., 1992; Daveby et al., 1998) by 7 – 30%. In another study, Carré et al. (1998) found that pea starch digestibility was higher for more finely ground pea. Starch digestibility in 3–wk–old broilers for pea with a particle size less than 100 µm, and greater than 100 µm was 95.7% and 84.4%; respectively. It was suggested that starch granule accessibility was restricted by the matrix of cell wall.

The hypothesis that pelleting may has positive effects on legume starch digestibility was first introduced by Moran et al. (1968) when they observed an improvement in pea ME value. This hypothesis was experimentally confirmed in several studies investigation effect of pelleting on pea or faba beans given to chickens or adult cockerels (Carré et al., 1987, 1991; Lacassagne et al., 1988; Conan et al., 1992; Grosjean et al., 1999). It was also reported that if the diets are pelleted, fine grinding is not needed to improve legume starch digestibility (Conan et al., 1992). Carré and Melcion, (1995) reported that starch digestibility of legume seeds is affected by thermo–mechanical processes. For instance, increasing the length of the dies or the flow rate may result in increased digestion of the starch.

The gelatinization of starch due to pelleting probably concerns only the outer part of pellets, since this part reaches high temperatures during the pelleting process. However, when the process is performed in dry conditions, high temperatures are

required to reach gelatinization in whole pellets, probably near 120 degrees (Colonna and Champ, 1990), however, this is not practical for pelleting feed. Moreover, during milling and grinding of feedstuffs, mechanical gelatinization of starch can occur. Friction that developed during grinding can also result in increased temperature of ingredients, which might gelatinize starch in some situations (Pesti et al., 2005).

In summary, the digestibility of pea nutrients is as a result of an interaction between access possibility and enzyme susceptibility. The low starch digestibility observed among coarse ground pea is as a result of a combination of access possibility and enzyme susceptibility. The difference between legume seeds and cereal grains in digestibility was found also with in vivo studies in poultry (Yutste et a1., 1991; Carré et al., 1998). The low digestibility of pea starch can be attributed to the other factors beside the starch granules structure (Carré, 2004).

2.4.4.3. Effects of Feed Processing on Protein Digestibility of Pea

Both grinding and pelleting have been shown to impact the digestibility of pea protein. However, the impact of grind size on the pea protein digestibility has not been consistent. Daveby et al. (1998) studied the effect of particle size on protein digestibility when dehulled–pea was milled (68% of particles < 670 µ) or crushed (32% of particles < 670 µ). The ileal protein digestibility was lower in chickens fed crushed diets than milled diets. Conan et al. (1992) and Daveby et al. (1998) failed to see and effect of In contrast, finer grinding of pea (1.5 vs 0.5 mm screen size) improved protein digestibility in chickens (70.2 and 89.5% respectively) with the effect thought to be to increasing protein susceptibility to enzyme hydrolysis (Crevieu et al., 1997). Protein digestibility of corn–pea and wheat–pea based diets (spring pea cultivars) was improved by pelleting, but the

effect was not related to the level of ANFs in the pea cultivars (Carré et al., 1991). The interaction effects of grinding and pelleting have not been studied but it can be speculated that pelleting may eliminate any effects of grinding such as is the case with starch digestion (Conan et al., 1992).

Moreover, protein digestibility is affected by pea cultivars. It was found that APD for yellow, green, and brown seeds of pea were 75.2, 72.8, and 60.4%, respectively in young broilers (Igbasan and Guenter, 1996a).

The form of diet fed to poultry can affect nutrient digestibility, body weight gain, feed intake, and feed conversion ratio. Plavnik (1997) reported that the growth rate of broilers and turkeys fed pelleted diets was higher than those fed mash diets, however, abdominal fat was increased in both species. The effect of feeding mash, pellet, and crumble feed forms on broilers performance was investigated by Jahan et al. (2006). They found that the mash form had the lowest body weight, the crumble form had the highest body weight, and the pellet form was intermediate. Lemme et al. (2006) found that broilers ate less mash feed than pelleted feed. Pelleting has also been shown to reduce the relative length of the digestive tract (Amerah et al., 2007). This improvement in chicken performance is more likely attributed to the other advantages of pelleting rather than the increased the nutrient digestibility of the diet.

Pelleting and grinding effects could be explained through mechanical energy usage. For example when water is added to pea before grinding, a 20% improvement in starch digestibility resulted but an increase in usage of mechanical energy also occurred (References). Similar to pelleting, heating legume seeds by autoclaving or micronization

increases starch digestibility and AME_n value (Moran et al., 1968; Conan and Carré, 1989; Brenes et al., 1993; Igbasan and Guenter, 1996).

Canadian pea has a low level of ANFs, which could help feed producers minimize the use of high temperatures during feed processing (Hickling 2003). Moreover, it has been noted that the grinding process improves digestible energy and amino acid digestibility, as well as increasing the rate of starch digestion (Carré et al. 1991).

2.4.5. Effect of Pea Cultivar on Pea Nutrient Digestibility

Igbasan and Guenter (1996a) determined the energy value for three different pea market classes, yellow, green, and brown, using 14–day–old broiler chicks. Pea seeds were ground using 2.0–mm screen size and included at 500 g/kg of diet. The AME_n values were 2,508, 2,603, and 2,006 kcal/kg (DM) for yellow, green, and brown samples; respectively. In another trial, the AME_n values of yellow and green pea cultivars were significantly different, 2,747 and 2694 kcal/kg, respectively. Pea was included at 450 g/kg of test diets and fed to 21–day–old male broiler chicks (Igbasan and Guenter, 1996b). In another study, using the precision–feeding technique, Igbasan et al. (1997) measured the true metabolizable energy values of yellow, green and brown seeded pea fed to adult leghorn cockerels and found TME_n values were 2947, 2866, and 2771 kcal/kg (DM), respectively (Igbasan et al., 1997).

2.5. Starch Digestibility Measurements in Poultry

2.5.1. In vitro Method

The extent of starch digestion among feed ingredients is quite similar; however, the rate and site of starch digestion are different. The method of in vitro starch degradation, using a digestive tract model, can be used to mimic the in vivo hydrolysis of

starch. In the case of rapid digested starch (RDS), the total starch digestion is reached at an early incubation time. Whereas the slow digested starch (SDS) needs a longer time to reach the total extent of starch degradation. With the assumption that the passage rate of digesta through the small intestine is not affected by feed formulation, the in vitro time can be used to represent the different segments in the small intestine. It can be projected that SDS results in more starch digestion in the posterior parts of the small intestine (ileum) than RDS.

Englyst et al. (1992) first proposed using an in vitro method to assess starch digestion. It was established to mimic the human small intestine conditions. Based on that method, starch digestibility is fractionated into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). Starch is hydrolyzed using exogenous enzymes, and then the released glucose at different incubation time is measured by glucose oxidase method and starch is calculated by (glucose × 0.9). Starch digestibility is estimated using total starch and degraded starch at each of incubation time. The in vitro method is quick, reliable, inexpensive laboratory method that does not need an animal be sacrificed for determining starch digestibility.

2.5.2. In vivo Method

There are two in vivo methods that are often used to measure starch digestibility, total starch digestibility and ileal starch digestibility. In both methods, indigestible indicators such as acid insoluble ash, chromic oxide, and titanium oxide are commonly used with poultry. The main assumption in this digestibility evaluation is that the passage rate of the starch and the indicator through the gastrointestinal tract of poultry are the same.

The first technique is based on the total starch digestibility and is used to estimate the extent of starch digestion. It is determined using the analytical results of starch and indicator contents in the diet and the excreta. It represents the amount of starch that is absorbed from the total gastrointestinal tract. The calculation of total starch digestibility is based on the change between the starch–indicator ratio in the diet and excreta. However, this technique does not distinguish between the amount of starch that is absorbed as glucose from the small intestine, or the amount of starch that fermented in the hindgut and absorbed in form of VFA. Compared to other techniques, this technique is less complicated and birds are not sacrificed.

Ileal starch digestibility is often determined using the slaughter technique. An indigestible marker is used in this method. Chickens are killed at the end of the feeding trial, the small intestine is removed, and digesta samples are collected. The experimental diets and collected digesta are analyzed for total starch and indigestible marker content. Based on the ratio of starch to indigestible marker in the diet and digesta, starch digestibility is calculated for sections of the small intestine. Even though this technique eliminates the effect of the micro–flora in the hind gut on starch digestibility, birds are need to be sacrificed Digesta from the number of birds within a replication fed the same experimental diet are pooled in order to provide sufficient sample for analysis and to provide a broader representation of starch digestion. Because starch (or a nutrient) digestibility can be determined at different sections of the small intestine, the rate of starch digestion can be observed along the small intestine. This technique allows the differentiation of SDS, RDS and RS in an ingredient or diet. In general, published data on starch digestibility is not always complete; the technique that was used, animal type,

weight, and age, and feed processing such as mill type, screen sizes, and pelleting conditions are not described well in many cases.

2.6. Feeding Pea

Field pea (*Pisum sativum* L.) is suitable for use in the diets of many types of poultry. It contains moderate levels of crude protein and metabolizable energy and can be classified as an energy and protein source. Compared to corn and wheat, pea contains relatively high levels of lysine and low levels of methionine. Therefore based on feed formulation they can partially or completely counter these deficiencies. Moreover, the availability and low cost of crystalline amino acids will make it possible to balance practical diets for these amino acids. Pea samples can be variable in chemical composition and nutrient digestibility (Conan and Carré, 1989; Gatel, 1994; Igbasan and Guenter, 1996a). Variability is related, among other things, to cultivar, growing conditions, harvest, and storage (Igbasan et al. 1997a). There are three issues related to the nutritional value of pea for poultry: nutrient composition, processing, and level of inclusion in diets.

Field pea has been the subject of much research published during the last four decades, but this research does not all always draw the same conclusions. For example, there is considerable variation in the maximum recommended levels of pea to be used in poultry diets. Reasons for the differences between research trials can be many, but a key aspect can be attributed to the accuracy of pea nutritional specifications, mainly ME and AAs digestibility, for poultry for feed formulation. Adding to this inaccuracy is the fact that pea respond markedly to feed processing and many trials do not provide sufficient detail of the nature of feed processing used. Moreover, there may be nutritional benefits

of feeding pea that go beyond specific nutrient specifications. For example, slow digested starch (SDS) has been reported to enhance poultry performance (Weurding et al., 2003b), but this is not demonstrated by terminal SI or faecal digestibility estimates.

The round–seeded, white–flowered, spring varieties that are commonly grown in Canada contain less CP than wrinkled–seed, colored–flowered, winter varieties of pea that are grown in Europe. In contrast, winter pea has higher levels ANFs such as trypsin inhibitor compared to spring varieties (Conan and Carré 1989). Winter pea contains lower AME_n and APD than spring pea (Carré et al. 1991).

2.6.1. Feeding Pea to Broiler Chickens

The use of pea in broiler diets has been studied in different places in the world and much of this work has suggested 100 to 200 g/kg as the upper limit of pea inclusion (Moran et al., 1968; Castell et al., 1996; Igbasan and Guenter, 1996a,b; Fasina and Campbell, 1997; McNeill et al., 2004; Li et al., 2006; Gutierrez del Alamo et al., 2009; Nalle et al., 2010). A high level of pea inclusion was recommended early by Brenes et al. (1989). It was indicated that pea inclusion up to 800 g/kg improved broiler performance (7 – 28 d) compared to corn–soy isolate diets; however, oil was added only to pea diets in this experiment, and this may have had some impact. In another study, Brenes et al. (1993) found that the addition of pea up to 470 g/kg did not affect the performance of young broilers (10 – 17 d) compared to corn–soybean diet. Castell et al. (1996) studied the effect of feeding pea in broiler chicks (0 – 21 d) of age. Pea was included at 0, 230, 460, and 680 g/kg of diets. Only FCR was reduced at 230 g/kg of pea inclusion, but all performance measurements were reduced at 460 and 680 g/kg of pea inclusion. Igbasan and Guenter (1996a) reported that the inclusion of up to 200 g/kg of yellow–, brown–,

and green–pea in broiler diets (4 – 18 d) did not affect BWG, but FCR was reduced only for the brown–pea–based diet. However, 400 g/kg pea inclusion depressed BWG and FCR. When diets were formulated with 115% of NRC recommended AAs, BWG, FI, and FCR were equivalent to the control diet. Feeding steam–pelleted pea/canola blend was studied in mixed–sex–broiler chicks 0 to 40 d of age. BWG and FCR declined as the blend inclusion increased (0, 100, 200, and 300 g/kg). The experiment failed to determine the upper level of pea inclusion in broiler diets (Fasina and Campbell, 1997). Some Australian studies have confirmed the pea inclusion up to 300 g/kg in broiler diets (0 – 42 d) with no adverse effect on performance (Farrell et al. 1999).

In all previous studies, the effect of pea inclusion on broiler performance was discussed in term of nutrient availability and the presence of ANFs. In fact, the potential of feeding SDS from pea on broiler performance was first investigated by Weurding et al. (2003). They compared a diet containing 340 g/kg pea to a control diet formulated with tapioca and maize. BW and FCR at 38 d of age were improved in the pea–fed birds, 1823 vs. 1729 g and 1.73 vs. 1.77 respectively. It was concluded that feeding SDS from pea has improved energy and protein availability.

A few recent studies have documented the performance of broilers consuming pea–based diets (Cowieson et al., 2003; McNeill et al., 2004; McNeill et al., 2004; Meng and Slominski, 2005; Moschini et al., 2005; Diaz et al., 2006; Li et al., 2006; Czerwinski et al., 2010; Laudadio and Tufarelli, 2010). While results are somewhat inconsistent, it is obvious that broilers respond well to moderate levels of pea in both starter and grower rations.

A high level of pea inclusion in broiler diets did not maintain the same performance as control diets in most of the studies that have been done. The accurate response of broilers to the nutrient value of pea was not determined. In fact, the young chicks have the low digestibility of nutrients compared to adult birds (Rynsburger, 2009). Therefore, it may have an impact on bird performance in older ages. For example, only 79% of pea AAs was digested by young chicks, with lysine and methionine were 83 and 70%; respectively (Ravindran et al., 2005). It can be concluded that diet formulation on the basis of starch digestibility and digestible amino acids (lysine, methionine, threonine, and tryptophan) may have improved the performance of birds consuming the higher levels of pea inclusion.

2.6.2. Feeding Pea to Laying Hens

The upper level of pea inclusion in laying hens diets has been determined in many studies (Moran et al., 1968; Castanon and Perez–Lanzac, 1990; Ivusic et al., 1994; Castell et al., 1996; Igbasan and Guenter, 1997a; Igbasan and Guenter, 1997b; Perez–Maldonado, et al., 1999; Fru–Nji and Pfeffer, 2007). However, some drawbacks have been found regarding missing identification of pea processing in those experiments. For example, feed form, grind size, and adding exogenous dietary enzymes were not outlined clearly. As a result the available information about feeding pea to laying hens is not complete. Moreover, maximizing pea inclusion in laying hens diets require more information about pea cultivar, diet formulation, and feed processing. The nutrients levels of pea appear to be well suited to provide the requirements of laying hens. Research cited elsewhere in this review suggests that adult birds are able to utilize pea nutrients as they have a mature digestive system.

An early trial using Honegger Blond laying hens (20 to 40 wk of age) indicated that 375 g/kg of pelleted pea inclusion had no adverse effect on egg production but egg size was reduced in comparison to control diet (fishmeal based diet). However, the use of oat–based diets, the low level of ME in diets, and the over supplement of methionine were not applicable to modern laying hens (Davidson, 1980). Another experiment also demonstrated that the level of dietary pea inclusion affected average egg weight with reduced weight at dietary pea inclusion levels above 333 g/kg (Castanon and Perez–Lanzac, 1990). However, diets were not supplemented with methionine and that may had some impact on the results. When corn–soybean control diet was compared to 0, 148, 445, and 590 g/kg pea diets supplemented with soybean oil and fed to Single Comb White Leghorn (SCWL) laying hens (22 to 58 wk of age), only thinner egg shells were observed at the 590 g/kg pea inclusion level (Ivusic et al., 1994). Igbasan and Guenter (1997) did not find any adverse effects on laying hen performance of feeding up to 400 g/kg pea compared to feeding a corn–soybean meal based diet. However, feeding 600 g/kg pea reduced egg production and weight and impaired FCR. In a 40 wk trial (25 to 65 wk of age), 250 g/kg pea inclusion had no effect on hen performance (Perez–Maldonada et al. 1999). Research conducted in North American suggests that moderate levels of pea inclusion are suitable for use in laying hen diets. Of note, there are no published reports on the impact of SDS from pea on laying hens.

2.6.3. Feeding Pea to Broiler Breeders

To the best of our knowledge there is only one study that has examined the effect of pea inclusion on broiler breeder performance. Field pea replaced corn and soybean meal as the only source of protein and starch in starter diets, and replaced part of corn and

soybean meal in grower and breeder diets fed to ISA Vedette dwarf broiler breeder pullets. Bird performance (BWG, hen–day–egg–production, egg weight, fertility, and hatchability) was measured up to 46 wk of age. There were no significant different between diets. It was concluded that field pea could be used as alternative feed ingredient for dwarf breeders (Kill and Savage, 1992).

Feed in broiler breeders is severely restricted in order to reduce body weight, improve flock uniformity, delay sexual maturity, increase egg production, reduce the number of unsettable eggs, and increase livability (Katanbaf et al., 1989; Robinson et al., 1993; Chen et al., 2006). However, feed restriction is associated with marked changes in bird metabolism during feeding and subsequent fasting periods. Moreover, feed restriction may cause a physiological stress associated with bird hunger, which can become a welfare issue. In order to minimize the negative effects of feed restriction, different quantitative studies have been conducted (Robinson et al., 1992; Hudson et al., 2001; de Jong et al., 2003; Renema and Robinson, 2004; Tolkamp et al., 2005; Chen et al., 2006; de Beer et al., 2008, Ekmay et al., 2010).

In humans, after consuming a meal, hunger and satiety are controlled by physiological mechanisms that relate to the GI of starch–based food. It has been suggested that the rate of gut emptying, glucose absorption rate, and dietary fiber may promote satiety in humans. Legume starches provoke slow blood glucose responses (Björck et al., 2000). Therefore, feeding broiler breeder pullets with pea as source of SDS may affect metabolism and maintain performance.

EFFECTS OF FEED PROCESSING ON PEA NUTRIENT DIGESTIBLITY FOR POULTRY

3.0. NUTRIENT DIGESTIBILITY OF PEA AS AFFECTED BY HAMMER–MILL SCREEN–HOLE SIZE AND COLD–PELLETING IN BROILER CHICKENS

3.1. Abstract

Pea is an accepted ingredient in poultry feeding but information on the impact of feed processing on its nutritional value is not extensive. Therefore, a 2 × 2 factorial arrangement was used to study the effect of hammer–mill screen–hole size (3.2– and 6.4–mm) and feed form (mash and cold–pelleting) on the rate and extent of pea nutrient utilization in broiler chickens. Pea–based diets were fed from 14 to 21 d of age and included acid insoluble ash as a digestibility marker. Feces were collected on d 19 and 20 for determination of AME_n. Digesta samples were collected from the anterior and posterior of both the jejunum and ileum at 21 d of age to determine the rate and extent of starch and protein digestibility. Digesta samples of the posterior ileum were used to determine ileal digestible energy (**IDE**). Data were analyzed as a completely randomized design with 6 replicates per treatment. There were no significant interactions between treatments and therefore the results are presented as main effects. Finer grinding resulted in a higher diet IDE and AME_n ($P < 0.001$) than course grinding, but cold–pelleting had no effect on energy values. Neither starch nor protein digestibility was affected by screen–hole size at the anterior and posterior jejunum, and anterior proximal ileum. However, total starch ($P = 0.008$) and protein ($P = 0.01$) digestibilities at the posterior ileum were affected. In contrast, cold–pelleting increased the digestibility of protein in the posterior jejunum and anterior ileum, but not in the anterior jejunum and posterior ileum. Starch digestibility was increased by cold–pelleting in all portions of the small

intestine. Pea starch and protein were slowly digested along the gut as demonstrated by 15 to 22% of pea starch and 11 to 16% of pea protein being digested in the ileum. In conclusion, hammer–mill screen–hole size and cold–pelleting independently affected the IDE, AME_n, protein, and starch digestibility of pea for broiler chickens.

Key words: pea, screen–hole size, cold–pelleting, AME_n, starch digestibility

3.2. Introduction

Field pea (*Pisum sativum* L.) has a moderate level of energy and protein content compared with other feed ingredients (NRC, 1994; Igbasan et al., 1997). Pea grain contains low levels of sulphur amino acids but can be successfully included in poultry diets when supplemented with other sources of these amino acids (Gatel, 1994). Although the use of pea in poultry diets is a common practice in some parts of the world, particularly in Europe, the maximum dietary level of pea inclusion is still not well established. One of the reasons is the limited knowledge of the nutritional value of pea, and more specifically the effect of feed processing on nutrient digestibility.

Energy and protein are the most important nutrients that are considered in formulation of poultry diets (Classen and Stevens, 1995). Most of the metabolizable energy in poultry diets is provided by starch, and its digestibility can be strongly correlated with AME (Rogel et al., 1987; Wiseman et al., 2000). Furthermore, the rate and extent of starch digestion are affected by structural properties of starch as well as feed processing (Classen, 1996; Carré, 2004; Svihus et al., 2005). Therefore, poultry

nutritionists need to understand the impact of feed processing on the availability of nutrients, most importantly starch for which little information is available.

The most common poultry feed processing techniques are grinding and pelleting. As the particle size is decreased by grinding, the surface area exposed to digestive enzymes increases; therefore the nutrient digestibility is improved (Behnke, 1996). Pelleting involves application of heat, moisture, and pressure to feed over variable time periods. The positive effect of pelleting on animal performance is attributed to reduced feed wastage, minimized ingredient segregation and consequently decreased feed selection, more efficient energy and time for prehension, elimination of diet pathogens, starch gelatinization, and improved palatability (Behnke, 1996). Pelleting can also reduce somewhat the level of heat labile anti–nutritional factors (**ANFs**) in feedstuffs. Cold–pelleting refers to the manufacture of pellets by adding water to feed before forcing through a die without steam conditioning. However, the frictional resistance of feed during pelleting causes some heat (Svihus and Gullord, 2002). Cold–pelleting should have all the advantages of steam–pelleting except starch gelatinization and elimination of ANFs and pathogenic organisms. Relatively few studies have focused on the effect of screen–hole size and pelleting conditions on the nutritive value of pea (Carré et al., 1991, 1998; Lacassagne et al., 1991; Crévieu et al., 1997; Fasina et al., 1997; Daveby et al., 1998). However, to the best of our knowledge cold–pelleting has not been studied in this regard.

Longstaff and McNab (1987) reported that the starch digestibility and TME_n of pea fed to adult cockerels were improved by grinding (through 1–mm screen–hole size) comparing with whole pea grains (88.3 vs. 75.6%; 2,690 vs. 2,286 kcal/kg; respectively).

This improvement was suggested to be due to increased intra–cellular accessibility by digestive enzymes as a result of disruption of the cotyledon cell wall. The impact of fine grinding (contrasting mean particle size of 0.16– vs. 0.50–mm) on starch digestibility and AME_n of faba bean was examined by Lacassagne et al. (1991). They reported that starch digestibility and AME_n increased in 21–d–old broiler chickens as the mean particle size decreased. However, apparent protein digestibility was not affected. In contrast, fine grinding of pea (0.5–mm screen–hole size), improved apparent protein digestibility in broiler chickens compared with 1.5–mm (Crévieu et al., 1997). They concluded that enzyme access is the most limiting factor in protein digestibility of pea. Using 17–d–old chickens, Daveby et al. (1998) found that starch and protein digestibility of milled–dehulled pea was increased compared with crushed–dehulled pea; however, only starch digestibility was significantly improved. Carré et al. (1998) concluded that grinding improves digestibility of pea starch by disrupting the cellular structure of granules and increasing the surface area exposed to digestive enzyme action.

The positive effect of steam–pelleting on the AME value of pea was first reported by Moran et al. (1968). Carré et al. (1987) found that steam–pelleting improved AME_n, starch, and true protein digestibility of pea fed to adult cockerels, and that the effect of steam–pelleting on starch and protein digestibility of corn and wheat was less pronounced compared to pea. These results were confirmed later with adult and young chickens (Carré et al., 1991). Fasina et al. (1997) studied the effect of steam–pelleting on pea–canola (1:1) based diets fed to male broilers (17–d–old). Feed ingredients were ground using a hammer mill fit with a 1.5–mm screen–hole size and pelleting temperature was maintained at 80ºC. Protein digestibility and AME_n were higher for pelleted diets than

untreated diets. Grosjean et al. (1999) similarly found a positive effect of pelleting a pea diet on AME, starch, and protein digestibility using adult cockerels.

In summary, research has shown that feed technology treatments such as grinding and pelleting can increase the digestibility of pea. It was hypothesized that processing influences the kinetics and degree of pea nutrient digestibility by broiler chickens. The objective of this experiment was to establish the impact of hammer–mill screen–hole size and feed form (mash vs. cold–pelleting) on the metabolizable energy and the site and extent of starch and protein digestion of pea using 21–day–old broiler chickens.

3.3. Materials and Methods

The experimental procedure was carried out in accordance with the Guide to the Care and Use of Experimental Animals, Canadian Council on Animal Care (1993) and was approved by the Animal Care Committee of the University of Saskatchewan.

3.3.1. Birds and Housing

A total of 96 one–day–old male broiler chicks (Ross × Ross 308) were obtained from a local hatchery (Lilydale Hatchery, Wynyard, SK. Canada) and housed in battery cages (50 cm width, 85 cm length, 25 cm high) with wire mesh floors. The cages were equipped with a trough feeder and two cup drinkers. Room temperature was 35°C at d 0 and gradually decreased 2.8°C per week during the experiment. Day length was 23 h (30 to 40 lx) from 0 to 7 d of age and 20 h (10 to 15 lx) for the remainder of the experiment. Birds were provided ad libitum access to water and feed through the experiment. A conventional broiler starter (Corn–based crumble diet from Co–op Feeds, Saskatoon, SK. Canada) was fed from 1 to 14 d of age. At 14 d of age, birds were weighed on a cage

basis (4 birds per cage), cages were randomly assigned to one of the 4 dietary treatments (6 replicates per treatment), and fed experimental diets.

3.3.2. Experimental Diets

Experimental diets were formulated using pea (Eclipse, yellow cotyledon cultivar) as the only source of energy and amino acids. The ingredients and calculated nutrient profile of the experimental diet are presented in Table 3.1. Acid insoluble ash (**AIA**) (Celite Corporation, Quincy, WA, USA) was used as an indigestible marker to allow for the determination of nutrient digestibility. The digestible amino acid content of pea was estimated based on AminoDat 3.0 Platinum (2006). Pea grains were ground with a full circle pulverator hammer–mill (Model 160–D, Jacobson Machine Works, Minneapolis, MN 55427, USA) fitted with one of two screens–hole size (3.2–, 6.4–mm). Feed was mixed (Hobart mixer, Model L–800, Hobart Canada, Don Mills, ON M3B 1B1) in two batches, one for each grind size and then each grind size was split into two equal portions, one portion to be fed in mash form and the other to be cold–pelleted. For cold–pelleting, water (18% on a weight basis) was added to the diet and the diet was then pelleted using a small–scale meat grinder (Hobart grinder, Model N50, Hobart Canada, Don Mills, ON M3B 1B1) fitted with a 4.5–mm die. As a result of fractional resistance of feed, pellet temperature averaged approximately 60°C immediately after pelleting. Pellets were dried overnight in a forced air oven at 55°C. Pelleted diets were crumbled with a roller mill prior to feeding. Samples were collected from all diets for particle measurement and chemical analyses.

3.3.2.1. Diet Particle Size Distribution

The particle size distribution of experimental diets was determined by the laser diffraction method (Hetland et al., 2002) using a Malvern Mastersizer instrument with Hydro 2000G accessories (Malvern Instruments Ltd, Malvern, Worcestershire, UK) at the Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Aas, Norway. Wet sieving was performed on the mash and pelleted diets. Samples were soaked in distilled water and then wet–sifted, particles larger than 2–mm were later dried overnight at 104°C and weighed.

The particle size distribution of experimental diets was determined by the laser diffraction method (Hetland et al., 2002) using a Malvern Mastersizer instrument with Hydro 2000G accessories (Malvern Instruments Ltd, Malvern, Worcestershire, UK) at the Department of Animal Science, Agricultural University of Norway, Trondheim, Norway. Wet sieving was performed on the mash and pelleted diets. Samples were soaked in distilled water and then wet-sifted, particles larger than 2–mm were later dried overnight at 104°C and weighed.

3.4. Data Collection

Feed intake (FI) and body weight gain (BWG) were measured on a cage basis for the experiment period (14 to 21 d) and feed conversion ratio (FCR) was calculated for the same period. Energy retention (AME_n) was determined using fecal samples collected on d 19 and 20 while the rate and extent of starch and protein digestion were determined using collected small intestine digesta at 21 d of age. Mortality was recorded daily. Body weights of dead birds were used to correct the FCR calculation.

3.4.1. Excreta Collection

Feces were collected for 48 h at 19 and 20 d post–hatching. Clean excreta trays covered with plastic sheets were placed under each battery cage and excreta was collected every 12 h. For each excreta collection, feed and feather contaminants were removed and then excreta were placed in plastic bags and immediately frozen at –20°C. Subsequently samples were dried using a forced air oven (55°C), pooled from the same replicate, and ground using a Retsch (Model RM 200, Retsch Gmbh, Haan, Germany) laboratory mill (1.0–mm sieve–hole size).

3.4.2. Digesta Collection

The experiment was terminated on d 21 and birds were euthanized by cervical dislocation and the intestinal tract was removed. The small intestine was divided into four sections, anterior jejunum (AJ), posterior jejunum (PJ), anterior ileum (AI) and posterior ileum (PI). The jejunum and ileum sections were separated at Meckel's diverticulum and the posterior ileum was defined as the section half way between Meckel's diverticulum and 2 cm anterior to the ileal–cecal junction. The digesta content from each section of the small intestine was gently squeezed out (using a roller vial) directly into a 100 ml snap–cap vial. Digesta samples were pooled by replicate, held on ice during collection and then stored at –20^{o}C. Digesta samples were later freeze–dried in order to minimize bacterial growth and subsequent effects on nutrient content. After freeze–drying, the samples were finely ground with a mortar and pestle, and mixed thoroughly before analysis.

3.4.3. Chemical Analyses

Diets, excreta, and small intestine digesta were analyzed for dry matter, AIA, crude protein (N × 6.25), total starch, and gross energy (only samples from posterior

ileum). Moisture was determined using standard procedures of AOAC (1990) and AIA was determined using the procedure of Vogtmann et al. (1975). Gross energy was determined using an oxygen bomb calorimeter (Model 1281, Parr Instruments, Moline, IL, USA) standardized with benzoic acid. The crude protein content was analyzed by a Leco Protein Analyzer (Model Leco–FP–528L, Leco Corporation, St. Joseph, MA, USA). Total starch was determined using the Megazyme analysis kit (Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland) based on the use of thermostable α–amylase and amyloglucosidase (McCleary et al., 1997). The chemical analyses of all samples were performed in duplicate, except for total starch and AIA, which were analyzed in triplicate and quadruplicate, respectively.

3.4.4. Nutrient Retention Calculation

The gross energy (GE), nitrogen (N) and AIA content of diets, ileal digesta, and excreta were used to determine AME_n and apparent ileal digestible energy (IDE). Nitrogen correction values of AME were determined by correction for zero nitrogen retention as described by Hill and Anderson (1958). The following equations were used with appropriate corrections for differences in dry matter (DM) content:

$$AME_{n\ (cal/g.diet)} = AME_{cal/g.diet} - (8220 \times ANR_{g/g.diet})$$

$$AME_{cal/g.diet} = GE_{cal/g.diet} - [GE_{cal/g.excreta} \times (\%\ AIA\ diet \div \%\ AIA\ excreta)]$$

$$ANR_{g/g.diet} = N_{g/g.diet} - [N_{g/g.excreta} \times (\%\ AIA\ diet \div \%\ AIA\ excreta)]$$

Where:

$ANR_{g/g.diet}$ = Apparent Nitrogen Retained (g/g of diet)

8220 = Correction factor (cal) per g nitrogen retained in the body

The total starch, protein, acid insoluble ash data of diet and digesta were used to calculate the digestibility (%) of starch and protein in each part of the small intestine using the following equation:

Digestibility = $1 - [(\%\ AIA_{diet} \div \%\ AIA_{digesta}) \times (\%\ Nutrient_{digesta} \div \%\ Nutrient_{diet})] \times 100$

3.5. Statistical Analysis

The experimental design was a complete randomized design (CRD). Each experimental diet was fed to 6 replicates (cages) with 4 birds per replicate from 14 to 21 d of age. Data were subjected to two–way analysis of variance (2 screen–hole sizes, 3.2– or 6.4–mm × 2 feed forms, mash or cold–pelleted) using the general linear model (GLM) procedure of SAS 9.2 software (SAS, 2008). Data were checked for normality using the Shapiro–Wilk test prior to analysis. Treatment means were separated using Duncan's multiple range test and differences were considered significant when $P \leq 0.05$ unless otherwise stated. The statistical model used was $Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$, where Y_{ijk} is the observed parameter of an experimental unit in a cage k, and it that received a level i of factor α (screen–hole size), and a level j of factor β (feed form), μ the general mean, α the effect value of level i of screen–hole size (i = 1, 2), β the effect value of level j of feed form (j = 1, 2), $\alpha\beta$ the effect value of the interaction between level i of screen–hole size and level j of feed form, ε_{ijk} = The random error of an experiment unit $Y_{ijk} \cong$ NID $(0,\sigma^2\ \varepsilon)$.

3.6. Results

For all response criteria, the impact of hammer–mill screen–hole size and feed form (mash vs. cold–pelleting) were independent and no significant interactions were found between treatments. Therefore, only the main effects will be presented in this report.

3.6.1. Diet Particle Size Distribution

Particle size distributions of the experimental diets (Table 3.2) showed that screen–hole size of 6.4 mm increased the relative proportion of particles which were > 2000 µm (34.3 and 39.7% in mash and cold–pelleting diets, respectively) and reduced the proportion of fine particles, in comparison to the 3.2 mm screen–hole size. Cold–pelleting had a minor effect on particle size, but did increase the proportion of particles in the > 50 ≤ 350 µm category.

3.6.2. Growth Performance

The experiment was not designed to investigate the impact of hammer–mill screen–hole size and cold–pelleting on bird performance, but feed intake, body weight gain, and FCR were provided as information to define the experiment. Overall, performance criteria should be interpreted with caution because of the small number of birds used and the short growth period. Performance criteria were not affected by grind size or feed form (Table 3.3).

3.6.3. Energy Value

Energy retention was measured as IDE in the posterior ileum as well as the more traditional AME_n based on feces data. Finer grind size, 3.2 mm vs. 6.4 mm, increased energy utilization as assessed by both techniques (Table 3.4). In contrast, the values of energy retention of mash and pellet forms were not significantly different, although both IDE and AME_n increased numerically with cold–pelleting.

3.6.4. Starch Digestibility

Digestion of starch was consistently higher for the cold–pelleted than mash diets regardless of measurement location; starch digestion values for cold–pelleted diets were

~10% higher at all small intestine sections (Table 3.4). In contrast, screen–hole size did not affect digestibility in more anterior portions of the small intestine but by the PI, fine grinding (3.2 mm) resulted in higher digestibility in comparison with coarse grinding (6.4 mm). The results regarding pea starch digestibility are further demonstrated in Figures 3.2 and 3.3. At the PI, digestibility of pea starch was maximum (75.6%) with 3.2 mm hammer–mill screen–hole size and cold–pellet diets, whereas it was minimum (54.6%) with 6.4–mm hammer–mill screen–hole size and mash diets. It is also demonstrated that pea starch is slowly degraded along the small intestine of chickens. Less than 57% of pea starch was digested in the upper half of the small intestine for all four treatments, with total tract digestibility of approximately 70%.

3.6.5. Protein Digestibility

Cold–pelleting improved the apparent digestibility of pea protein in the PJ and AI sections of the small intestine in comparison to values for mash diets (Table 3.4). Apparent digestibility of protein was higher in PI for the 3.2 mm screen size treatment vs. the 6.4 mm grind size. Fecal protein digestibility was not affected by of screen–hole size but cold–pelleting reduced apparent fecal protein digestibility in comparison to mash form (36.3 vs. 43.5%).

3.7. Discussion

The present study was designed to examine the effects of feed processing on pea nutrient digestibility and not broiler performance. The performance data are reported to provide the context under which the experiment was completed. It is noted as well that the diets were not formulated to meet all broiler nutrient requirements and hence performance did not meet industry standards. The 7 to 14 d performance data (FI, BWG, and FCR) of broiler chickens were unaffected by screen–hole size or cold–pelleting, but demonstrate that the birds were eating feed and gaining weight during the experimental period.

The small intestine of chicken is the primary site of nutrient digestion and absorption and it can be further subdivided into the duodenum, jejunum and ileum. Readily digested nutrients are absorbed in the duodenum, but most absorption occurs in the jejunum (Leeson and Summers, 2001; Pesti et al., 2005). In nutrition, posterior ileum digestibility is more relevant than at other sections as it represents the extent of nutrient digestibility (Lemme et al., 2004). However, because digested nutrients in the small intestine are utilized first by the gut itself, the availability of nutrients along the small intestine would reduce the reliance of systemic nutrients for gut maintenance and function. Moreover, absorbed nutrients may elicit different metabolic responses, which may have some impact on animal performance (Weurding et al., 2003b). Because pea starch is classified as slowly digestible, it is of interest to understand the digestion location of pea nutrients (starch, protein) and how they are affected by screen–hole size and feed form. In most previous studies, the effect of screen–hole size on the rate and site of starch digestion was not examined.

The IDE and AME_n values of experimental diets were both improved by fine grind size (25 and 10% respectively). These results agree with earlier research by Longstaff and McNab (1987) who found that the energy value (TME_n) of ground pea was significantly higher than whole pea grain. Also, Carré et al. (1998) concluded that the AME of pea was improved in a diet with small particle sizes (< 100 µm) in comparison to a diet with large particle size (> 100 µm). The effect of particle size was not the same as with a wheat–soybean isolate based diet, as unground wheat improved nutrient utilization (Svihus et al., 2004). The effect of particle size was anticipated and likely reflects more ready access to starch and protein molecules by digestive enzymes in the broiler gastrointestinal tract. In contrast, cold–pelleting did not affect either energy determination technique (P = 0.54 for IDE and 0.09 for AME_n) compared to the mash diet. However, previous studies have shown that pelleting improves the energy utilization of pea in chickens (Moran et al., 1968; Carré et al., 1987, 1991; Grosjean et al., 1999). The lack of effect of pelleting likely relates to the use of a cold–pelleting procedure that does not apply heat or steam during the pelleting process. A numeric increase in energy retention (3.6 and 3.8% for IDE and AME_n; respectively) was noted for cold–pelleted in comparison to mash diets. The energy retention reflects the significant increase in starch and protein digestion.

Starch digestibility is affected by the amount and nature of the surface area exposed to digestive enzymes, which in turn can be related to factors such as granule size, degree of crystallinity, and the physiochemical structure of the starch itself (Moran, 1982). The maximum extent of pea starch digestibility (80.8%) was reached with the combination of 3.2 mm screen–hole size and cold–pelleting, whereas it was minimum

(59. 7%) with 6.4 mm screen–hole size and mash diet. Screen–hole size did not affect anterior jejunum digestibility indicating no effect on the proportion of rapidly degraded starch, but smaller particle size (3.2 mm screen–hole size) resulted in increased starch digestibility by the end of the ileum. The effects of screen–hole size on starch digestion in the small intestine support the mechanism of increased surface area for enzymatic action. These results also reflect the reported effect of screen–hole size on IDE and AME_n. The data also confirm the results of previous research on the effect of grinding on the extent of starch digestion of pea (Longstaff and McNab, 1987; Carré et al., 1991, 1998; Daveby et al., 1998).

Eliasson and Gudmundsson (2006) showed that starch gelatinization occurs at a temperature range between 45 to 90°C, depending on the moisture content and source of starch. Only 5 to 20% of starch is gelatinized when it is steam pelleted (Svihus et al., 2004). Therefore, the cold–pelleting procedure applied in this experiment is not expected to be associated with starch gelatinization. Despite an expected lack of effect on gelatinization, starch digestibility was improved by approximately 10% by cold–pelleting at the four locations of the small intestine assessed. This suggests that cold–pelleting is altering digestibility by an effect other than gelatinization. The increase in terminal ileal starch digestibility agrees with the results of other studies completed with steam pelleting at high temperature (Moran et al., 1968; Carré et al., 1987, 1991; Grosjean et al. 1999).

The results confirmed that pea starch is slowly degraded along the small intestine of chickens (Weurding et al., 2001). Across all treatments, only 48.8 to 58.9% of pea starch was digested in the upper half of the small intestine (AJ and PJ) with a total tract digestibility of around 70%. A fraction of pea starch appears to be readily digested as

shown by the AJ values and the level of this starch is impacted by the cold–pelleting process, 28.2 vs. 40.0% for mash and cold–pelleted diets, respectively. As noted above, this fraction is not affected by fineness of grind. The impact of feed form is seen throughout the small intestine sections as digestibility curves for the two feed forms remained parallel for each grind size.

Finer grinding resulted in numerically higher protein digestibility values for the more anterior portions of the small intestine, but the difference only became significant in the posterior ileum. Correspondingly, Daveby et al. (1998) reported the positive effect of small screen–hole size on apparent ileal protein digestibility of pea. They suggested that access to the intra–cellular structure and the cell walls are the causes for the low digestibility. Cold–pelleting resulted in higher digestibly in the PJ and AI but did not affect digestibility in other portions of the small intestine, i.e., it affected the site of protein digestion but not the extent. Previous studies have shown that steam pelleting improved pea protein digestibility (Carré et al., 1991). This experiment suggests that cold–pelleting does not cause thermal modification of protein. The data also show that 11 to 16% of pea protein was digested in the ileum.

In conclusion, the nutritive value of pea can be improved using proper processing methods. The impacts of hammer–mill screen–hole size and feed form (mash vs. cold–pelleting) on pea nutrient digestibility were independent. The results clearly indicate that pea starch is slowly digested in the small intestine with 15 to 22% of the starch digested in the ileum, and those strategies to alter starch digestion kinetics and total starch digestion might be different. Further research is needed in to establish the effect of feed processing on pea nutritional value and more precisely on starch digestibility.

TABLE 3.1. The composition and calculated nutrient contents of the experimental diets fed from 14 to 21 d of age

Ingredient	%
Pea	89.71
Canola oil	5.00
Dicalcium phosphate	1.28
Ground limestone	1.52
Sodium chloride	0.39
Vitamin–mineral premix[1]	0.50
Choline chloride 70%	0.10
Celite–insoluble ash[2]	1.50
Nutrient, calculated	
ME (kcal/kg)	2,768
Crude protein	21.27
Starch	43.50
Calcium	0.95
Non–phytate P	0.42
Crude fat	6.12
Chloride	0.28
Potassium	0.99
Sodium	0.18
Linoleic acid	1.75
Arginine	1.26
Lysine	1.44
Met + Cys	0.43
Threonine	0.85
Tryptophan	0.21

[1]Vitamin–mineral premix provided the following per kilogram of complete diet: vitamin A, 11000 IU; vitamin D, 2200 IU; vitamin E, 30 IU; vitamin K_3, 2 mg; biotin, 0.15 mg; niacin, 60 mg; pyridoxine, 4 mg; riboflavin, 6.0 mg; thiamine, 1.5 mg; pantothenic acid, 10 mg; folic acid, 0.6 mg; vitamin B_{12}, 0.02 mg; copper, 10 mg; manganese, 80 mg; iron, 80 mg; zinc, 80 mg; iodine, 0.8 mg; and selenium, 0.3 mg.
[2] Celite Corporation, Quincy, WA, USA.

TABLE 3.2. Distribution of particle size in experimental diets (%)

Hammer–mill screen–hole size	3.2–mm		6.4–mm	
Feed form	Mash	Cold–pellet	Mash	Cold–pellet
Particle size (µm)				
> 0 ≤ 50	21.56	14.64	11.81	9.24
> 50 ≤ 350	16.30	38.68	11.75	23.79
> 350 ≤ 500	4.41	6.03	1.89	5.60
> 500 ≤ 650	10.99	10.46	5.76	9.59
> 650 ≤ 800	10.22	7.38	6.54	5.80
> 800 ≤ 1000	11.28	6.53	8.36	4.06
> 1000 ≤ 1350	10.49	4.70	8.78	1.89
> 1350 ≤ 1750	7.84	2.64	7.17	0.31
> 1750 ≤ 2000	3.77	1.02	3.64	0.00
> 2000	3.14	7.92	34.30	39.72

TABLE 3.3. Effect of hammer–mill screen–hole size and feed form on performance of broiler chickens (14 to 21d post–hatching)

Parameters	Hammer–mill screen size (mm)			Feed form			SEM[1]
	3.2	6.4	*P*	Mash	Cold–pellet	*P*	
FI[2] (g/bird)	517[3]	528	NS	506	539	NS	11.1
BWG[2] (g/bird)	270	257	NS	256	270	NS	6.7
FCR[2] (g/g)	1.92	2.08	NS	2.00	1.99	NS	0.0423

[1]SEM pooled.
[2] FI–feed intake; BWG–body weight gain; FCR–feed conversion ratio.
[3] Each value represents the mean of 12 replicates with 4 birds each.

TABLE 3.4. Effect of hammer–mill screen–hole size and feed form on pea nutrient digestibility

Parameters	Screen–hole size (mm)			Feed form			SEM[1]
	3.2	6.4	*p*	Mash	Cold–pellet	*p*	
Energy (kcal/kg DM)							
IDE[2]	2675[4 a]	2135[b]	< 0.001	2363	2447	NS	85.2
AME_n[2]	2637[a]	2405[b]	< 0.001	2474	2568	NS	36.3
Starch digestibility (%)							
AJ[3]	33.1	35.1	NS	28.2[b]	40.0[a]	< 0.001	1.89
PJ[3]	53.8	50.5	NS	47.4[b]	57.0[a]	0.009	1.86
AI[3]	61.4	56.2	NS	49.9[b]	67.7[a]	< 0.001	2.50
PI[3]	70.1[a]	59.7[b]	0.008	59.9[b]	70.2[a]	0.007	2.25
Protein digestibility (%)							
AJ	32.9	23.4	NS	31.0	25.3	NS	4.61
PJ	70.1	66.3	NS	64.3[b]	72.1[a]	0.005	1.47
AI	77.4	74.8	NS	72.3[b]	79.8[a]	< 0.001	1.11
PI	82.4[a]	77.1[b]	0.010	78.8	80.8	NS	1.09
Excreta	39.3	40.5	NS	43.5[a]	36.3[b]	0.002	1.20

[a, b] Means within a main effect with different superscripts are significantly different ($P < 0.05$).
[1]Pooled SEM.
[2] IDE–ileal digestible energy (kcal/kg DM); AME_n (kcal/kg DM).
[3] AJ–anterior jejunum; PJ–posterior jejunum; AI–anterior ileum; PI–posterior ileum.
[4] Each value represented the mean of 6 replicates (4 birds per replicate).

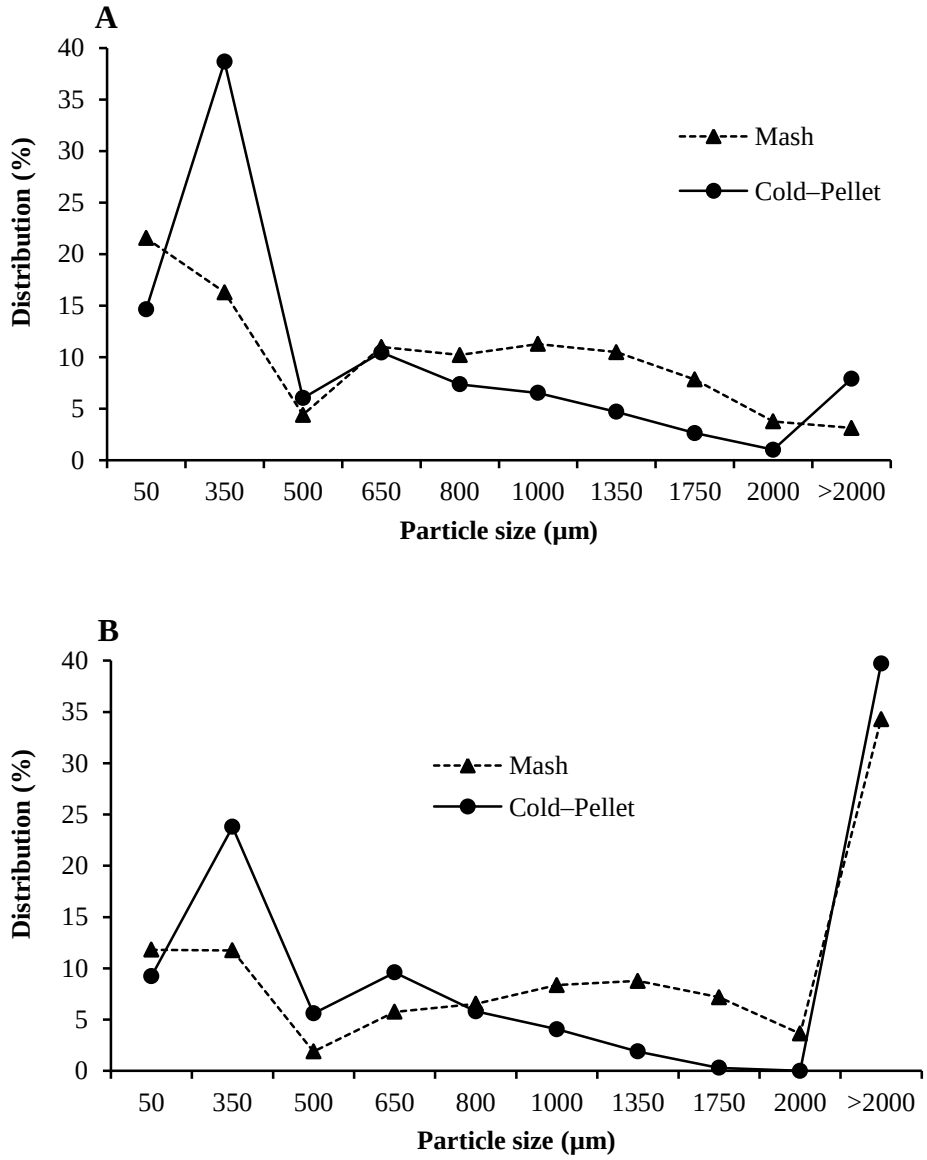

FIGURE 3.1. Particle size distribution (%) of pea–based diets ground using 3.2–mm (A) and 6.4–mm (B) hammer–mill screen–hole size diets.

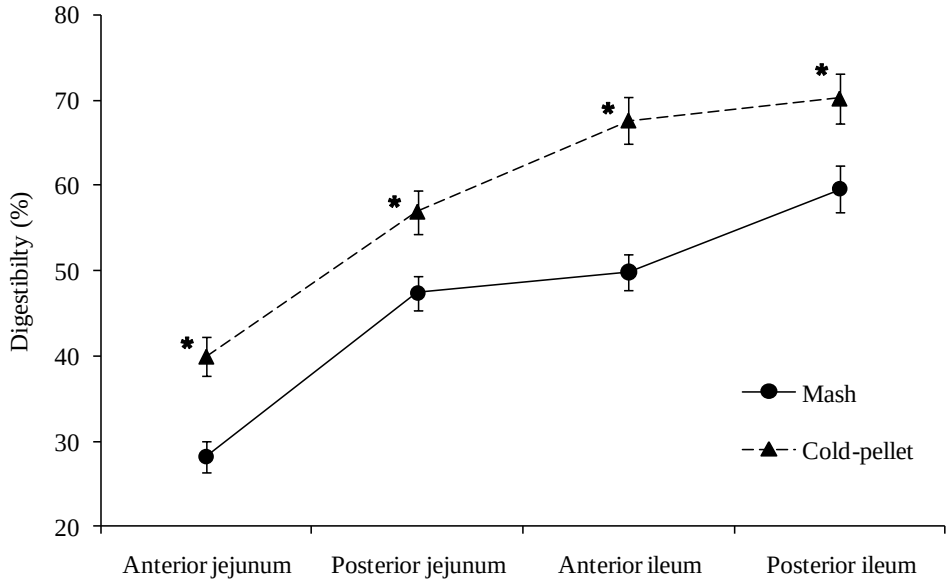

FIGURE 3.2. Effect of feed form (mash and cold–pelleting) on starch digestion of pea fed to broilers (21 d). Each data point is the mean of 12 observations. Bars represent SEM and an asterisk (*) indicates sections for which a significant ($P \leq 0.05$) difference was found between feed forms.

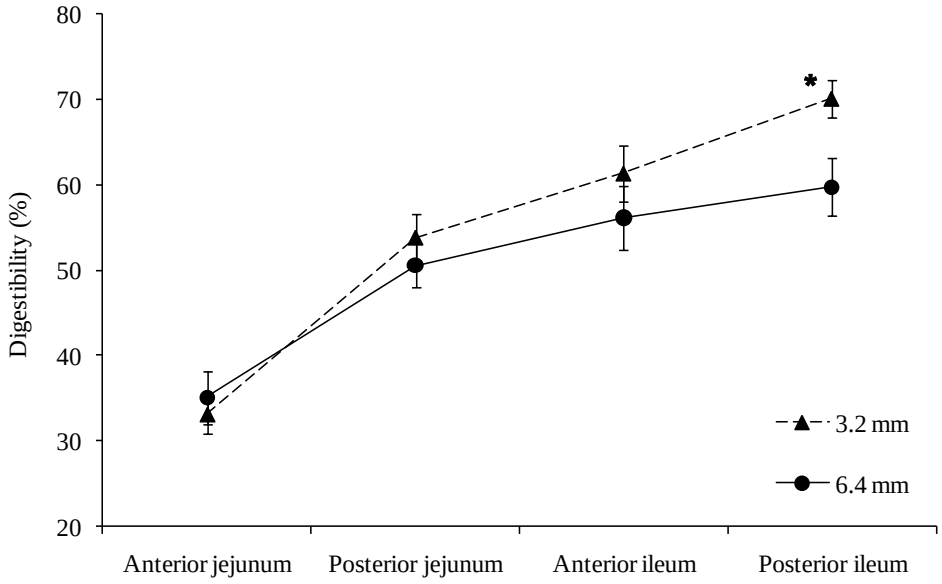

FIGURE 3.3. Effect of hammer–mill screen–hole size (3.2– and 6.4–mm) on starch digestion of pea fed to broilers (21 d). Each data point is the mean of 12 observations. Bars represent SEM and an asterisk (*) indicates sections for which a significant ($P \leq 0.05$) difference was found between screen–hole sizes.

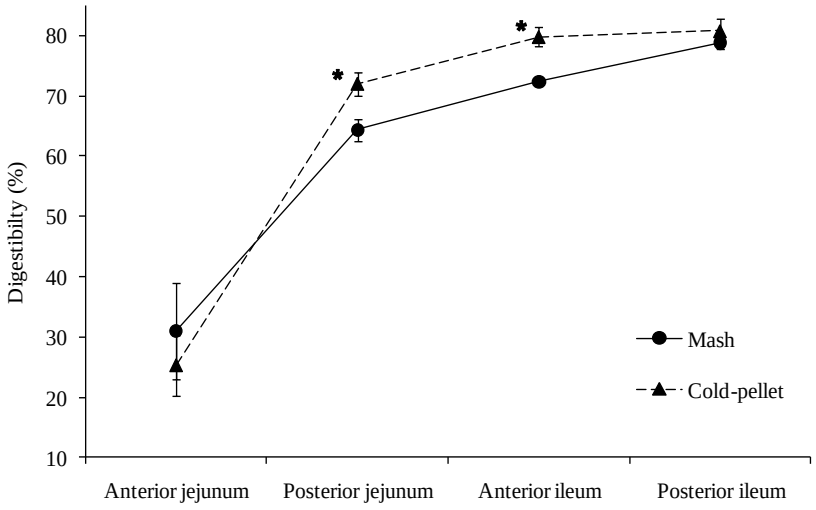

FIGURE 3.4. Effect of feed form (mash and cold–pelleting) on pea protein digestibility in broilers (21 d). Each data point is the mean of 12 observations. Bars represent SEM and an asterisk (*) indicates sections for which a significant ($P \leq 0.05$) difference was found between feed forms.

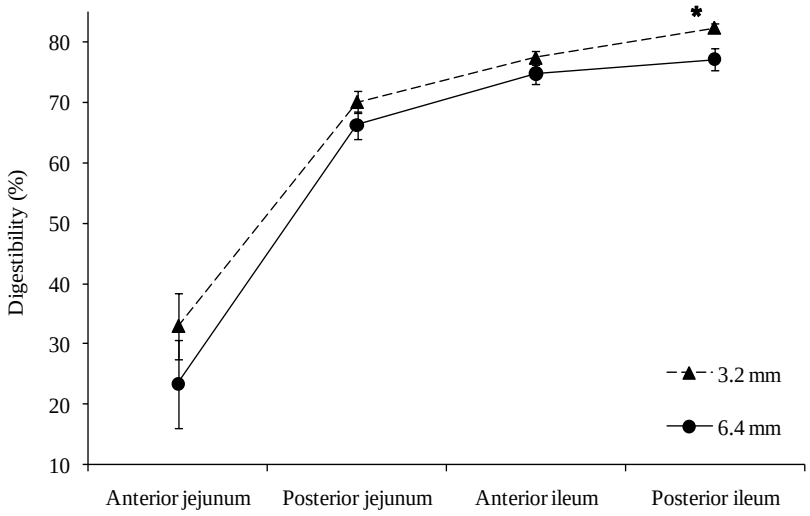

FIGURE 3.5. Effect of hammer mill screen–hole size (3.2– and 6.4–mm) on pea protein digestibility fed to broilers (21 d). Each data point is the mean of 12 observations. Bars represent SEM and an asterisk (*) indicates sections for which a significant ($P \leq 0.05$) difference was found between screen–hole sizes.

4.0. NUTRIENT DIGESTIBILITY OF PEA AS AFFECTED BY HAMMER–MILL SCREEN–HOLE SIZE AND PRE–PELLETING CONDITIONING TEMPERATURE IN BROILER CHICKENS

4.1. Abstract

Pea is an accepted ingredient in poultry feeding, but information on the impact of feed processing on its nutritional value is not extensive. Therefore, a 2 × 5 factorial arrangement was used to study the effect of hammer–mill screen–hole size (3.2– and 6.4–mm) and pre–pelleting conditioning temperature (60, 70, 78, 85, and 92°C) on AME, AME_n, and the rate and extent of starch and protein digestibility of pea in broiler chickens. Pea–based diets, including acid insoluble ash as a digestibility marker, were fed from 14 to 21 d of age. Excreta were collected on d 19 and 20 for AME determination. Birds were killed on day 21 and digesta samples were collected from the anterior and posterior sections of both the jejunum and ileum to determine the site, rate, and extent of starch and protein digestibility. Data were analyzed as 2 × 5 factorial with 6 replicates per treatment and 6 birds per replication. There were no interactions ($P > 0.05$) between hammer–mill screen–hole size and pre–pelleting conditioning temperature. The 3.2–mm screen–hole size increased pea AME, AME_n, and protein digestibility, but did not affect starch digestion in comparison to diets containing pea ground using the 6.4–mm screen–hole size. Pre–pelleting conditioning temperature affected AME, AME_n, and starch, and protein digestibility. Energy retention was affected in a quadratic fashion with the highest value achieved at 70°C. Starch digestibility increased with increasing temperature in the anterior jejunum, but decreased with increasing temperature in the posterior ileum. Protein digestibility decreased with increasing temperature in the posterior jejunum,

anterior ileum, posterior ileum and excreta. In conclusion, hammer–mill screen–hole size and pre–pelleting conditioning temperature have important but independent effects on the feeding value of pea with finer grind and a pre–pelleting conditioning temperature of 70°C resulting in the highest digestibility.

Key words: AME, starch, protein, pea, feed processing

4.2. Introduction

Pea (*Pisum sativum* L.) is grown in many parts of the world and is used for both human food and animal feed. Pea has a high level of lysine, but it is deficient in sulphur–containing amino acids (Hickling, 2003). Nonetheless, the cost and availability of crystalline amino acids have made it possible to overcome this deficiency and formulate poultry diets containing pea by supplementing them with DL–methionine (Gatel, 1994). In general, pea has a moderate level of energy and protein and can be used in poultry diets (NRC, 1994; Igbasan et al., 1997).

Starch is an important nutritional component of pea and its characteristics impact the rate and extent to which it is digested by poultry. Most pea starch granules have an oval shape and range in diameter from 2 to 40 µm. In contrast cereal grains have starch granules less than 19 µm in diameter. Large granule size, which results in a smaller granule surface area, has been associated with lower digestibility than what is found for small starch granules (Oates, 1997; Tester et al., 2006). The surface of pea starch granules is smooth and in contrast to most cereal starches, there is no evidence of fissures, pin–holes or compound granules that might provide access to amylase during

starch hydrolysis. Field pea starch contains up to 49.6% amylose while cereal starches contain less than 25%. Starch hydrolysis is influenced by the ratio of amylose to amylopectin and starches with high amylopectin (e.g. waxy) content are digested more rapidly than those high in amylose. Moreover, surface protein encapsulation of starch granules, and the packing of the amylopectin double helices (C–type) and crystallinity (18.9 to 36.5%) also impact starch digestibility (Ratnayake et al., 2002; Hoover and Zhou, 2003; Jane, 2004; Wang and Daun, 2004; Eliasson and Gudmundsson, 2006; Lehmann and Robin, 2007). As a consequence of the above characteristics, pea starch is more slowly digested and less digestible than cereal grain starches in poultry (Yutste et al., 1991; Weurding et al., 2001).

Processing techniques are applied to modify the structure of feedstuffs and improve nutritional value in poultry diets, and grinding and pelleting are by far the most common feed processing techniques. The particle size of feed ingredients can be reduced by grinding and as the particle size is decreased, the surface area exposed to digestive enzymes increases, and as a result, nutrient digestibility is improved (Behnke, 1996; Pesti et al., 2005). Steam pelleting combines the effects of heat, moisture, pressure, and time. It has been applied historically in poultry diets in order to improve feed efficiency through decreased ingredient segregation, feed selection, feed wastage, pathogenic organism growth, level of anti–nutritional factors (ANFs), and energy expended for prehension (Behnke, 1996). Pelleting may also cause starch gelatinization, which can improve starch digestibility (Behnke, 1996; Pesti et al., 2005). As a result of high conditioning temperatures, pelleting can also have adverse nutritional effects such as destruction of vitamins and enzymes, reducing amino acid digestibility, and increasing the formation of

resistant starch (Rooney and Pflugfelder, 1986; Pickford, 1992; Thomas et al., 1998; Silversides and Bedford, 1999).

Feed processing has important effects on the nutritional value of pea and other similar legumes, and is an important consideration when they are used in poultry feed. Longstaff and McNab (1987) reported that starch digestibility and TME_n of pea fed to adult cockerels were improved by grinding (through 1 mm sieve) in comparison to feeding whole pea seed. This digestibility improvement was explained by increased intra–cellular enzyme accessibility as a result of grinding interrupting the cotyledon cell wall. Lacassagne et al. (1991) studied the effect of grinding on faba bean nutrient digestibility and found starch digestibility and AME were significantly increased as particle size decreased. Fine grinding of pea seed (0.5 mm screen–hole size) improved apparent protein digestibility comparing to coarse grinding (1.5 mm screen–hole size) in broiler chickens. It was concluded that susceptibility to enzyme hydrolysis is the most limiting factor in the digestibility of pea protein (Crevieu et al., 1997). Carré et al. (1998) concluded that grinding could improve digestibility of starch by disrupting the cellular structure of granules, which increases the surface area. Daveby et al. (1998) also found that starch and protein digestibility of pea was improved in chickens by reducing particle size; however, only starch digestibility was significantly affected.

In poultry, the positive effect of pelleting on the AME value of pea was reported early by Moran et al. (1968). Carré et al. (1987) found that steam pelleting improved the AME_n, and starch and protein digestibility of pea fed to adult cockerels. These results were confirmed later with adult and young chickens (Carré et al., 1991). Fasina et al. (1997) found that the AME_n and protein digestibility of steam pelleted (80ºC) pea–canola

(1:1) based diets were higher than for untreated mash diets (ground using a hammer–mill fit with 1.5 mm screen size). Grosjean et al. (1999) also confirmed the positive effect of pelleting pea on AME, starch, and protein digestibility using adult cockerels. In summary, considerable evidence exists for the positive benefit of grinding and pelleting on pea digestibility. Despite this research, questions remain on the interactive effects of grinding and pelleting, and the pelleting temperature required to optimize nutrient digestibility.

Therefore, the objective of this experiment was to establish the effect of hammer–mill screen–hole size and pelleting–conditions temperature on pea AME and the site and extent of pea starch and protein digestion in broiler chickens. It was hypothesized that grinding and pelleting influence the site and extent of pea nutrient digestibility by broiler chickens in an independent manner.

4.3. Materials and Methods

The experimental procedure was approved by the Animal Care Committee of the University of Saskatchewan. It was conducted in accordance with the Guide to the Care and Use of Experimental Animals, Canadian Council on Animal Care (1993).

4.3.1. Birds and Housing

A total of 360 one–day–old male broiler chicks (Ross × Ross 308) were obtained from a commercial hatchery (Lilydale, Wynyard, SK. Canada) and housed in battery cages (50 cm width, 85 cm length, and 25 cm high) with wire mesh floors. The cages were equipped with a trough feeder and two cup drinkers. The experimental room was environmentally controlled and temperature was 35°C at d 0 and gradually decreased 2.8°C per week during the experiment. Day length was 23 h (30 to 40 lx) from 0 to 7 d of

age and 20 h (10 to 15 lx) for the remainder of the experiment. Birds were provided ad–libitum access to water and feed during the course of the experiment. A conventional broiler starter diet was fed from 1 to 14 d of age. At 14 d of age, birds were weighed on a cage basis (6 birds per cage), cages were randomly assigned to one of 10 dietary treatments (6 replications per treatment), and fed experimental diets.

4.3.2. Experimental Diets

Experimental diets were formulated using pea as the only source of starch and the main source of amino acids; the only other protein source was DL–methionine. Pea (Eclipse, yellow cotyledon, developed by Limagrain, The Netherlands) was provided by the Crop Development Centre, University of Saskatchewan. The ingredients and calculated nutrient profile of the experimental diets are presented in Table 4.1. Acid insoluble ash (**AIA**) (Celite Corporation, Quincy, WA, USA) was used as an indigestible marker to determine nutrient digestibility. Pea amino acid content was calculated based on Degussa (Feed Additives, AminoDat® 3.0 Platinum (2006). Whole pea seeds were ground in a full circle pulverator–hammer mill (Model 160–D, Jacobson Machine Works, Minneapolis, Minn. 55427, USA) fitted with one of two screen–hole sizes (3.2–, 6.4–mm). Afterward, feed ingredients were mixed using a bakery mixer (Hobart mixer, Model L–800, Hobart Canada, Don Mills, ON. M3B 1B1) in two batches, one for each grind size and then each grind size was split into five equal batches. Each batch was conditioned and pelleted under different temperature conditions. The measured conditioning temperatures for each diet are presented in Table 4.2. The different conditioning temperatures were obtained by adjusting the steam pressure. As the steam pressure increased, the flow rate of steam increased and as a result the temperature

increased. Pelleted diets were crumbled with a roller mill prior to feeding. Samples were collected from all diets for particle size measurement and chemical analyses.

4.3.2.1. Pelleting Process

Diets were pelleted using a double pass conditioner pellet mill unit (CPM–Laboratory pellet mill, Model CL–5, California Pellet Mill Company, Crawfordsville, Indiana, USA) at the Department of Agricultural and Bioresource Engineering, University of Saskatchewan. The pelleting system consists of a receiving hopper, a vibratory feeder controlling flow of feed into the conditioners, two conditioners (102.7 mm inside diameter and 830 mm length each), a steam supply line connected to the upper conditioner, and a pelleter with a rotating ring die and stationary roller. The ring die was 4.5 mm in diameter and 45 mm in length. Conditioning and conditioning temperature were adjusted by regulating steam pressure and adjusting the speed of the mixing paddle. Conditioning temperatures (°C) were measured and recorded through stiff thermocouples in the upper and lower conditioners (3 stiff thermocouples in each), the pelleter feeder, and post–pelleting at the outlet. The conditioning and pelleting temperatures were recorded in a computer using Pelletmon software (Pelletmon, Steam pelleter monitor / Datlogger program, Version 2.07, September 1997). Pelleted feed was cooled and dried by spreading on trays and using forced air for 20 min at ambient temperature. All diets were stored at room temperature until the experiment was conducted. To maintain the desired conditioning temperature and production rate for each diet, a portion of each pea–based diet was conditioned and pelleted in order to warm up pelleter parts before treatment pelleting occurred. Representative samples (500 g/diet) were taken for proximate and other analyses.

4.3.3. Diet Particle Size Distribution

The particle size distribution of experimental diets was determined by the laser diffraction method (Hetland et al., 2002) using a Malvern Mastersizer instrument with Hydro 2000G accessories (Malvern Instruments Ltd, Malvern, Worcestershire, UK) at the Department of Animal Science, Agricultural University of Norway, Trondheim, Norway. Wet sieving was performed on the mash and pelleted diets. Samples were soaked in distilled water and then wet–sifted, particles larger than 2–mm were later dried overnight at 104°C and weighed.

4.3.4. Data Collection

4.3.4.1. Performance Data

Feed intake (**FI**) and body weight (**BW**) were recorded on a cage basis at d 14 and d 21, and weight gain (**BWG**) and feed conversion ratio (**FCR**) were calculated for the 14–21 d period. Mortality was recorded during the course of the experiment. Body weights of dead birds were used to correct the FCR calculation.

4.3.4.2. Excreta Collection

Feces were collected for 48 h at 20 and 21 d of age. Clean excreta trays covered with plastic sheets were placed under each battery cage and excreta was collected every 12 h (4 collections to minimize changes in excreta composition). For each excreta collection, feed and feather contaminants were removed and then excreta were placed in plastic bags and immediately frozen at –20°C. Subsequently samples were dried using a forced air oven (55°C), pooled from the same replicate and treatment, and ground using a centrifugal laboratory mill (Retsch Mortar grinder RM 200, Newtown, PA 18940, USA)

fit with 1.0 mm screen–hole size. Analysis of fecal samples was completed for the determination of energy retention (AME and AME_n).

4.3.4.3. Digesta Collection

The experiment was terminated on d 22 and birds were killed by cervical dislocation and the intestinal tract was removed. The small intestine was divided into four sections: Anterior jejunum (**AJ**), posterior jejunum (**PJ**), anterior ileum (**AI**) and posterior ileum (**PI**). The jejunum and ileum sections were separated at Meckel's diverticulum and the posterior ileum was defined as the section half way between Meckel's diverticulum and approximately 2 cm anterior to the ileal–cecal junction. Both the jejunum and ileum were split into two parts of equal length defined as anterior and posterior. The digesta content from each section of the small intestine was gently squeezed out (using a roller vial) directly into 100 ml snap–cap vial. Digesta was pooled by replicate and treatment, and during collection samples were held on ice. Digesta samples were stored after completing every replicate at –20°C and later freeze–dried. Digesta samples were freeze–dried in order to minimize bacterial growth and subsequent effects on nutrient content. After freeze drying, the samples were ground with a mortar and pestle and mixed thoroughly before analysis. Collected digesta samples were used to determine the site and extent of starch and protein digestion.

4.3.5. Chemical Analyses

Samples from diets, excreta, and small intestine digesta were analyzed for dry matter, gross energy, total starch, nitrogen (protein = N × 6.25), and AIA. Moisture was determined using standard procedures (AOAC, 1990) and gross energy was determined using an oxygen bomb calorimeter (Model 1281; Parr Instruments, Moline, IL)

standardized with benzoic acid. Total starch was determined colorimetrically using the Megazyme analysis kit (Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland) based on the use of thermostable α–amylase and amyloglucosidase (McCleary et al., 1997). The nitrogen content was analyzed by a Leco–FP–528 protein analyzer (Model 601–500–100, Serial # 3211, Leco Corporation, St. Joseph, MA, USA). AIA was determined using a modified version of the procedure from Vogtmann et al. (1975). In summary, approximately 1–2 g of sample is weighed into a borosilicate tube (16 × 125 mm) and ashed at 500°C for 24 h or until the sample turns to white ash. Following the ashing, 5 ml of 4N HCl is added and mixed thoroughly by vortexing, covered with glass marbles and then heated at 120°C for an hour. After that, tubes are centrifuged at 2500 x g for 10 minutes. The supernatant is aspirated and the precipitant washed repeatedly with water. The sample is dried overnight at 80°C and the dried sample is ashed again at 500°C overnight. The chemical analyses of all samples were performed in duplicate except for total starch that was completed in triplicate and diet AIA that was done in quadruplicate.

4.3.6. Calculations

The gross energy (GE), nitrogen (N) and AIA content of diets and excreta were used to determine AME and nitrogen corrected apparent metabolizable energy AME_n using the following equations with appropriate corrections for differences in dry matter (DM) content:

$$AME_{n\ (cal/g.diet)} = AME_{cal/g.diet} - (8220 \times ANR_{g/g.diet})$$

$$AME_{cal/g.diet} = GE_{cal/g.diet} - [GE_{cal/g.excreta} \times (AIA\%\ diet \div AIA\%\ excreta)]$$

$$ANR_{g/g.diet} = N_{g/g.diet} - [N_{g/g.excreta} \times (AIA\%\ diet \div AIA\%\ excreta)]$$

Where GE is gross energy, N is nitrogen, AIA is acid insoluble ash, $ANR_{g/g.diet}$ is apparent nitrogen retained (g/g of diet), and 8220 is correction factor (cal) per g nitrogen retained in the body (Hill and Anderson, 1958).

The total starch, protein, acid insoluble ash data of diet and digesta were used to calculate the digestibility (%) of starch and protein in each part of the small intestine sections using the following equation:

$$\text{Digestibility} = 1 - [(AIA\%_{diet} \div AIA\%_{digesta}) \times (Nutrient\%_{digesta} \div Nutrient\%_{diet})] \times 100$$

4.3.7. Statistical Analysis

The experiment was conducted as a complete randomized design (CRD). Each experimental diet was fed to 6 replicates (cages) with 6 birds per replicate from 14 to 21 d of age. All data were subjected to two–way analysis of variance (2 screen–hole sizes 3.2– or 6.4–mm × 5 pelleting–conditioning temperatures) using the Mixed procedure of SAS 9.2 (2008). Data were checked for normality using PROC Univariate test of SAS prior to analysis. Treatment means were separated using Tukey's Studentized Range Test. Differences were considered significant if $P \leq 0.05$ unless otherwise stated. Linear and quadratic polynomial contrasts were used to determine the effect of conditioning temperature. The statistical model used was $Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$, where Y_{ijk} is the observation of an experimental unit in a cage *k*, that received a level *i* of factor α (screen–hole size), and a level *j* of factor β (pelleting–conditioning temperature). μ = General mean; α = effect value of level *i* of screen–hole size, i = 1, 2; β = effect value of level *j* of pelleting–conditioning temperature, j = 1, 2, 3, 4, 5; $\alpha\beta$ = effect of the interaction between level *i* of screen–hole size and level j of pelleting–conditioning temperature; ε_{ijk} = The random error of an experiment unit $Y_{ijk} \cong$ NID $(0,\sigma^2 \varepsilon)$.

4.4. Results

For all response criteria, the impacts of hammer–mill screen–hole size and pre–pelleting conditioning temperature were independent and no interactions were found between fixed effects. Therefore only the main effects are presented in this study.

4.4.1. Diet Particle Size Distribution

The distributions of particle size for the experimental diets are presented in Table 4.3. Grinding pea using the 6.4 mm screen–hole size reduced the relative proportion of < 500 µm particles by 12.9% and increased the relative proportion of > 2000 µm particles by 14.3%. Increasing the pelleting–conditioning temperature increased the relative proportion of > 2000 µm particles and reduced the proportion of fine particles (< 500 µm) in diets ground using both screen–hole sizes, but the effect was larger for pea ground using the 6.4 mm screen–hole size. For instance, as the pelleting–conditioning temperature increased from 63 to 92ºC, the relative proportion of particles > 2000 µm increased from 7.7 to 12.9% in diets ground using 3.2 mm screen–hole size and from 16.3 to 27.2% in diets ground using 6.4 mm screen–hole size. The proportion of particles > 500 µm and < 2000 µm were almost identical for the two screen–hole sizes regardless of pelleting–conditioning temperature.

4.4.2. Bird Performance

The growth performance of broiler chickens is presented in Table 4.4. Fine grinding (3.2 mm screen–hole size) resulted in higher BW at 21 of age, BWG, and better FCR compared to coarse grinding (6.4 mm screen–hole size); however, feed intake was not affected by screen–hole size. Pelleting–conditioning temperature affected FI, BW,

and BWG, but FCR was not affected. However, there were no clear patterns or regression analysis effects of pelleting–conditioning temperature on these effects.

4.4.3. Nutrient Retention

4.4.3.1. Energy value

Finer grind size, 3.2– vs. 6.4–mm screen–hole sizes, increased AME and AME_n value by approximately 6% (Table 4.5). Pelleting–conditioning temperature affected energy value in a quadratic manner with the highest AME and AME_n for 70°C and the lowest values after pelleting at 92°C. The quadratic equations describing the relationship between pelleting–conditioning temperature and energy retention are presented in Table 4.6.

4.4.3.2. Starch Digestibility

Neither the site nor the extent of starch digestion was affected by screen–hole size (Table 4.6). Starch digestion values for both screen–hole sizes were 59% by the posterior part of jejunum. Based on a posterior ileal digestibility of approximately 80%, around 20% of starch was digested and absorbed in the ileum and half of that amount was digested by the end of the anterior ileum.

Pelleting–conditioning temperature affected starch digestibility in the anterior jejunum as well as at the posterior ileum. In the anterior jejunum, digestibility increased with pelleting–conditioning temperature while at the posterior ileum, the reverse was true. Numerically, the conditioning–pelleting temperature of 70°C had the highest starch digestion (86.1%) whereas the highest pelleting–conditioning temperature (92°C) had the lowest starch digestion (79.1%).

4.4.3.3. Protein Digestibility

Grinding through the small screen–hole size (3.2 mm) improved apparent protein digestibility over all sections of the small intestine as well as in the excreta (Table 4.7). Apparent protein digestibility was improved by an average of 4%. Pelleting–conditioning temperature affected the apparent protein digestibility in the posterior section of the jejunum as well the anterior and posterior portions of the ileum. Apparent protein digestibility was negatively affected by increasing conditioning–pelleting temperatures in all sections. The highest pelleting–conditioning temperature (92°C) had the lowest apparent protein digestibility in the posterior jejunum, anterior and posterior ileum, and excreta. The effects of experimental treatments on apparent protein digestibility are further demonstrated in Figures 4.5 and 4.6.

4.5. Discussion

The small intestine of chicken is the primary site of nutrient digestion and absorption. Even though most nutrient digestion and absorption occurs in the proximal part of the small intestine, the extent of nutrient digestion is usually determined at the distal end of the ileum (Lemme et al., 2004). However, because digested nutrients in the small intestine are utilized first by the gut itself, the availability of nutrients along the small intestine would reduce the reliance of systemic nutrients for gut maintenance and function (Wu, 1998). Because pea starch is slowly digested (Weurding et al., 2001, Ebsim et al., 2013), it is of our interest to understand the digestion location of pea nutrients (starch, protein) and how it is affected by particle size (hammer mill screen–hole size) and pelleting–conditioning temperature.

The performance data are presented to provide context to the experiment. The diets were not formulated to meet all broiler nutrient requirements and hence performance did not meet industry standards. The 14–21 d performance data (FI, 21 d BW, BWG and FCR) of broiler chickens were affected by screen–hole size and pelleting–conditioning temperature. Broiler performance was improved by feeding pea ground through a small screen–hole size (3.2 mm) and the 70°C pelleting–conditioning temperature had the best 21 d BW, BWG, and FCR. For the most part the production data match nutrient digestibility results, in that maximum performance and digestibility were achieved by the same treatments.

Wet–sieving of the experimental diets showed differences in particle size distribution due to hammer mill screen–hole size and pelleting–conditioning temperature. As expected, over all pelleting–conditioning temperature, the 6.4 mm screen–hole size resulted in larger particle sizes with 22.4% of particles being over 2 mm compared with 9.4% for the 3.2 mm screen–hole size. Correspondingly, the coarse diets had 51.2% of particles < 500 µm compared with 62.7% in the fine diet. As the pelleting–conditioning temperature increased the proportion of large particles increased. The reason for the larger particles at higher temperatures may be due to decreased friction during the pelleting process. The addition of more steam to the conditioner to raise temperatures may have lessened friction during feed passage through the pellet die, and thereby resulted in less particle size reduction. Although not statistically analyzed, the data indicate that pea ground using the 6.4 mm screen–hole size was more affected by the pelleting–conditioning temperature. Because the particle size was larger for this treatment, it is logical that it might be more affected.

The AME value of pea has been reported to range from 2600 to 3200 kcal/kg (Longstaff and McNab, 1987; Carré et al., 1991; Igbasan and Guenter, 1996; Grosjean et al., 1999; Perez–Maldonado et al., 1999; Nalle et al., 2011). Pea cultivar, methodology of assay, bird type and age, and feed processing may be responsible for this variation. The AME values of pea determined in this experiment (2352–2713 kcal/kg) for broiler chickens are similar to previous results reported by Igbasan and Guenter (1996).

The AME and AME_n values of pea were improved by small screen–hole size. These results agree with earlier research by Longstaff and McNab (1987) who found that the energy value (TME_n) of ground pea was significantly higher than whole pea grain. Carré, et al. (1998) also concluded that the AME of pea was improved by fine grinding. Of interest, the effect of grinding has not been reported for other grains, as particle size has been shown to have no effect on nutrient utilization (Svihus et al., 2004). The effect of grinding fineness was anticipated and likely reflects greater access to starch and protein molecules by digestive enzymes in the broiler gastrointestinal tract.

Pelleting–conditioning temperature affected the energy value of pea in a quadratic manner with 70°C resulting in the highest energy value. Previous studies that have shown that pelleting improves the energy value of pea in chickens (Moran et al., 1968; Carré et al., 1987, 1991; Grosjean et al., 1999), but this is the first study to document the response of pea to a graded range of pelleting–conditioning temperature and that higher temperatures reduce pea energy value. Chemical changes during pelleting affect starch and protein digestibility (Peisker, 2006) and it appears that pea is more susceptible to the negative effects of high temperature. It is possible that starch is retrograded and

indigestible starch–protein and starch–lipid complexes are formed (Creswell and Bedford, 2006).

Higher values for AME were reported in experiments by Carré et al., (1991) with 19 d of age broiler (3015 kcal/kg), Perez–Maldonado et al., (1999) with non–laying adult hens (3061 kcal/kg). Recently, Nalle et al. (2010) using adult broiler had reported 2939 kcal/kg.

Starch, the major energy–yielding source in commercial poultry diets, is nutritionally classified into rapidly digested (RDS), slowly digested (SDS), and resistant starch (RS) (Englyst et al., 1992). RDS is digested completely by the end of the jejunum and SDS digested by the end of the small intestine whereas RS escapes digestion in the small intestine and may be fermented in the ceca or large intestine. Moreover, the rate and extent of starch digestion are affected by the structural properties of starch granule and feed processing (Moran, 1982; Classen, 1996; Carre, 2004; Svihus et al., 2005). Starch digestibility is affected by the amount and nature of the surface area, which in turn can be related to factors such as granule size, degree of crystallinity, and the nature of the starch structure itself. In most previous studies, the effects of screen–hole size, conditioning temperature, and their interaction on the rate and extent of pea starch digestion were not examined.

The results so far clearly indicate that the pea starch is slowly digested in the small intestine with 19 to 26% of the starch digested in the ileum. The maximum pea starch digestibility (86.1%) was reached at 70°C pelleting–conditioning temperature, whereas it was minimum (79.1%) at 92°C pelleting–conditioning temperature. Only 60% of pea starch is digested in the anterior small intestine (AJ and PJ) with total tract

digestibility of around 82%. A considerable fraction of pea starch appears to be readily digested as shown by the AJ values.

It was hypothesized that the smaller hammer–mill screen–hole size would increase starch digestion based on increased surface area for enzymatic action. Surprisingly, no effect of screen–hole size on pea starch digestibility was found for any small intestine section, unlike what we have previously reported in Chapter 3 and has been shown by others (Longstaff and McNab, 1987; Carré et al., 1991, 1998; Daveby et al., 1998). In Chapter 3, no differences in starch digestibility were noted in the anterior jejunum due to grind size, but the cumulative starch digestibility increased more rapidly for the finely ground pea and resulted in a significantly different terminal ileum digestibility coefficient. Although not significant for the current experiment, a similar trend was seen for the impact of screen–hole size on starch digestibility with virtually identical values in the anterior jejunum and the 3.2 mm screen–hole size treatment producing 2.2% higher starch digestion at the posterior ileum. Although there was no interaction between main effects, pelleting–conditioning temperature may have altered the effect of screen–hole size and reduced the significance of its effect. This may partially relate to the smaller particle size found for the lower conditioning–pelleting temperatures and the observation that pea ground using a 6.4 mm screen–hole size appeared to be more affected than the 3.2 mm screen–hole treatment. Nutritionally, grinding disrupts the seed coat, starch granule, and protein matrix, which increases surface area and improves nutrient digestibility and energy utilization. Also, during pelleting as starch molecules are heated in the presence of water, starch gelatinization occurred. It results in disruption in the crystalline structure and increased swelling and solubility of starch granules (Singh et

al., 2010). However, the lack of screen–hole size effect on starch digestibility may be due to the effect of pelleting equipment (ring die was 4.5 mm in diameter and 45 mm length). As the particle size of coarse screen–hole size (6.4 mm) reduced and that may have evened out the differences in particle sizes. This was also suggested by Amerah et al. (2008).

Increasing pelleting–conditioning temperature resulted in a linear increase in anterior jejunum starch digestibility. This finding suggests that the increasing pelleting–conditioning temperature made more of the starch rapidly available to digestive enzymes. In contrast, by the posterior ileum, starch digestion decreased with increasing pelleting–conditioning temperature. This supports a negative impact on starch digestibility as a result of an increase in resistant starch. The starch digestion data for pelleting–conditioning temperatures follows the same trend as pea AME.

Eliasson and Gudmundsson (2006) showed that starch gelatinization occurs at a range of temperature (45 to 90°C) depending on the moisture content and source of starch. The native starch granules are inaccessible to enzymatic hydrolysis; therefore gelatinization may increase susceptibility of starch for amylolytic hydrolysis. Therefore, the pelleting–conditioning temperature procedure applied in this experiment may have induced some starch gelatinization. The increase in distal ileal starch digestibility agrees with the results of other studies completed with steam pelleting at high temperature (Moran et al., 1968; Carré et al., 1987, 1991; Grosjean et al. 1999). The moderate conditioning temperature (70°C) resulted in starch gelatinization and destruction of cell wall, therefore nutrient availability improved.

Protein digestibility can be determined using digesta or excreta analysis. However, protein digestibility at the posterior ileum is more reliable than excreta analysis. It takes into account the amount of endogenous (basal and specific) and unabsorbed amino acids. The amount of specific endogenous amino acids is diet–related and in the case of feeding pea, a significant endogenous loss has been reported in pigs (Leterme et al., 1996). Variations in protein digestibility of pea have been reported in poultry (Brenes et al., 1993; Igbasan and Guenter, 1996; Igbasan et al., 1997; Grosjean et al., 1999); therefore, the nutritive value of pea may be underestimated and pea inclusion in poultry diets minimized.

Small screen–hole size (3.2 mm) consistently resulted in higher protein digestibility values for all small intestine sections. This finding indicates that fine grinding provided more access to proteins for digestion and that course grinding did not. However, only 8.7% of protein was digested in the distal part of the small intestine. Conditioning–pelleting temperature affected protein digestibility in the PJ, AI, and PI but did not affect digestibility in AJ section. In other words, it affected the site and the extent of protein digestion. Previous studies have shown that steam pelleting improves pea protein digestibility (Carré et al., 1991). However, data from this experiment suggest that higher pelleting–conditioning temperature causes a negative thermal modification of protein because protein digestibility decreased as temperature increased. In general, it is thought that pelleting improves protein digestibility by denaturing protein and thereby increasing digestive enzyme accessibility. However, the negative effect of pelleting–conditioning temperature on protein digestibility may be related to Maillard reaction, destruction of heat–labile amino acids, and formation of starch–protein complexes. The

latter explanation may have more merit in this study because changes in protein digestibility with increasing conditioning–pelleting temperature match changes in starch digestibility.

The average of ileal protein digestibility reported in the current study (74%) was lower than previous studies, which may be related to assay methodology and pea cultivar. For instance, Bandegan et al. (2011) reported 86.1% digestibility of pea protein. In the present study, pea was included at a level of 89% and diets were balanced only with DL–methionine. Therefore, an imbalanced amino acid profile may have increased endogenous amino acids losses and that may have affected the value of protein digestibility.

In summary, the present study demonstrated that the nutritive value of pea can be improved by particle size reduction and pelleting. Fine grinding (3.2– vs 6.4–mm screen–hole size) improved both energy and protein digestibility. Beneficial effects of pelleting–conditioning temperature on nutrient digestibility were maximized at approximately 70°C. It is further demonstrated that the impacts of hammer–mill screen–hole size and pelleting–conditioning temperature on pea nutrient digestibility are independent.

TABLE 4.1. Ingredient composition and calculated nutrient content of the experimental diets

Ingredients	g/kg
Pea	834.6
Canola oil	80.0
Limestone	20.6
Di–calcium phosphate[1]	34.5
Sodium chloride	4.7
Vitamin–Mineral premix[2]	5.0
Choline chloride (60%)	1.0
DL–Methionine	4.6
Celite–insoluable ash[3]	15.0
Calculated nutrient content	
AME[4] (kcal/kg)	2,932
Crude protein (N × 6.25)	195.0
Total starch	367.4
Calcium	16.3
Non–phytate phosphorus	8.1
Sodium	2.1
Digestible Arginine	12.4
Digestible Lysine	11.7
Digestible Methionine	6.3
Digestible sulphur amino acids	7.7
Digestible Threonine	7.0
Digestible Tryptophan	1.7

[1]Di–calcium Phosphate: 15% Ca; 21% P.
[2]Supplied per kilogram of diet: vitamin A (retinyl acetate + retinyl palmitate), 11000 IU; vitamin D, 2200 IU; vitamin E (dl–alpha–topheryl acetate), 300 IU; menadione, 2.0 mg; thiamine, 1.5 mg; riboflavin, 6.0 mg; niacin, 60 mg; pyridoxine, 4.0 mg; vitamin B_{12}, 0.02 mg; pantothenic acid, 10.0 mg; folic acid, 0.6 mg; biotin, 0.15 mg; Iron, 80 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.8 mg; selenium, 0.3 mg; and $CaCO_3$, 500 mg.
[3]Celite Corporation, Quincy, WA, USA.
[4]National Research Council (1994).

TABLE 4.2. Measured conditioning and pelleting–conditioning temperature (°C) for each experimental diet

Treatment #	Screen–hole size (mm)	Min	Max	Average[1]	Average[2]
1	3.2	61.2	64.0	62.6	62.7
2	6.4	61.9	63.4	62.7	
3	3.2	69.0	71.9	70.1	70.0
4	6.4	68.0	71.4	69.8	
5	3.2	75.7	81.9	78.3	78.9
6	6.4	77.0	82.2	79.5	
7	3.2	83.9	86.9	85.3	84.8
8	6.4	83.0	84.6	84.3	
9	3.2	90.1	93.5	92.2	92.4
10	6.4	91.6	93.2	92.6	

[1]Average of each screen–hole size.
[2]Average of both screen–hole sizes.

TABLE 4.3. Relative proportion of particle size distribution in experimental diets (%)

Pelleting–conditioning temperature (°C)	63		70		79		85		92	
Hammer–mill screen–hole size (mm)	3.2	6.4	3.2	6.4	3.2	6.4	3.2	6.4	3.2	6.4
Particle size (µm)										
< 50	23.3	26.3	26.1	19.8	19.3	18.0	19.9	14.8	17.3	11.4
50–350	30.0	24.3	29.0	23.4	29.5	25.1	29.9	28.0	36.0	26.8
350–500	8.7	6.4	11.7	6.0	10.8	7.4	9.5	8.0	12.5	9.9
500–650	7.3	4.9	7.4	5.1	7.0	5.5	6.9	5.0	7.0	6.2
650–800	7.9	5.9	7.0	6.8	6.8	6.2	7.7	5.2	6.2	6.3
800–1000	6.9	6.1	5.8	7.8	7.1	6.2	7.2	4.7	4.4	5.5
1000–1350	4.5	5.0	3.0	7.2	6.1	5.1	5.6	3.5	2.3	4.0
1350–1750	3.0	4.1	1.2	4.9	3.9	3.2	3.2	2.0	0.8	2.2
1750–2000	0.7	0.8	0.5	1.2	0.9	0.8	0.7	0.6	0.4	0.5
> 2000	7.7	16.3	8.1	17.8	8.6	22.6	9.5	28.0	12.9	27.2

TABLE 4.4. Effect of hammer–mill screen–hole size and pelleting–conditioning temperature on growth performance of broiler chickens (14 to 21 d of age) fed pea–based diets

Parameters	Screen–hole size[1] (mm)		Conditioning–pelleting temperature[2] (°C)					SEM[3]
	3.2	6.4	63	70	79	85	92	
Feed intake (g/bird)	724	723	703^{bc}	726^{ab}	679^{c}	741^{ab}	769^{a}	6.5
21 d Body weight (g/bird)	808^{a}	774^{b}	790^{ab}	816^{a}	749^{b}	798^{ab}	804^{a}	6.9
Weight gain (g/bird)	389^{a}	358^{b}	371^{ab}	390^{a}	349^{b}	379^{ab}	381^{a}	4.3
FCR[4] (g/g)	1.853^{b}	2.046^{a}	1.873	1.867	1.935	2.044	2.028	0.0272

[a–c] Means in a row within screen–hole size and pelleting–conditioning temperature not sharing a common superscript are different ($P < 0.05$).
[1]Each value represents the mean of 30 replicates with 6 birds for each screen–hole size.
[2]Each value represents the mean of 12 replicates with 6 birds for each conditioning–pelleting temperature.
[3] SEM–pooled standard error of the mean (n = 60).
[4] FCR–feed conversion ratio was corrected for mortality by using the gains of the dead birds in the calculation.

TABLE 4.5. Effect of hammer–mill screen–hole size and pelleting–conditioning temperature on metabolizable energy (kcal/kg of DM) of pea fed to broiler chickens (14 to 21 d of age)

Parameters	Screen–hole size[1] (mm)		Conditioning–pelleting [2] (°C)					SEM[3]
	3.2	6.4	63	70	79	85	92	
AME (Diet)	2,782[a]	2,659[b]	2,796[a]	2,834[a]	2,758[ab]	2,682[b]	2,533[c]	19.2
AME_n (Diet)	2,632[a]	2,514[b]	2,644[a]	2,684[a]	2,608[ab]	2,538[b]	2,391[c]	18.5
AME (Pea)	2651[a]	2503[b]	2668[a]	2713[a]	2621[ab]	2530[b]	2352[c]	23.0
AME_n (Pea)	2471[a]	2329[b]	2485[a]	2533[a]	2442[ab]	2358[b]	2182[c]	22.2

[a–c] Means in a row within screen–hole size and pelleting–conditioning temperature not sharing a common superscript are different ($P < 0.05$).
[1]Each value represents the mean of 30 replicates with 6 birds for each screen–hole size.
[2]Each value represents the mean of 12 replicates with 6 birds for each conditioning–pelleting temperature.
[3]SEM–pooled standard error of the mean (n = 60).

TABLE 4.6. Effect of hammer–mill screen–hole size and pelleting–conditioning temperature on kinetic of starch digestion (%) of pea–based diets fed to broiler chickens (14 to 21 d of age)

Dietary treatments		Small intestine segments			
		Anterior jejunum	Posterior jejunum	Anterior ileum	Posterior ileum
Screen–hole size[1] (mm)	3.2	34.7	59.7	71.6	83.2
	6.4	34.7	59.3	69.9	81.0
Conditioning–pelleting temperature[2] (°C)	63	27.7[b]	58.3	71.2	82.9[ab]
	70	34.9[a]	61.1	72.5	86.1[a]
	78	34.2[ab]	58.3	70.2	80.7[b]
	85	36.1[a]	59.3	68.7	81.7[ab]
	92	40.7[a]	60.6	71.0	79.1[b]
SEM[3]		0.93	0.80	0.61	0.70

[a–c] Means in a column within screen–hole size and conditioning–pelleting temperature not sharing a common superscript are different ($P < 0.05$).
[1]Each value represents the mean of 30 replicates with 6 birds for each grind size.
[2]Each value represents the mean of 12 replicates with 6 birds for each pelleting–conditioning temperature.
[3] SEM–pooled standard error of the mean (n = 60).

TABLE 4.7. Effect of hammer–mill screen–hole size and pelleting–conditioning temperature on protein digestion (%) of pea–based diets fed to broiler chickens (14 to 21 d of age)

Treatments		Small intestine segments				Excreta
		Anterior jejunum	Posterior jejunum	Anterior ileum	Posterior ileum	
Screen–hole size[1] (mm)	3.2	47.6[a]	68.9[a]	74.4[a]	75.5[a]	58.7[a]
	6.4	44.0[b]	65.3[b]	71.8[b]	73.6[b]	56.6[b]
Conditioning–pelleting temperature[2] (°C)	63	44.6	70.5[a]	76.0[a]	76.6[a]	59.0[a]
	70	49.1	69.2[a]	74.7[ab]	76.3[a]	59.0[a]
	78	48.2	66.4[ab]	72.6[bc]	74.1[ab]	58.0[ab]
	85	42.7	66.2[ab]	72.0[bc]	74.1[ab]	56.2[b]
	92	44.4	63.2[b]	70.2[c]	71.6[b]	55.9[b]
	SEM[3]	0.91	0.69	0.48	0.45	0.36

[a–c] Means in a column within screen–hole size and pelleting–conditioning temperature not sharing a common superscript are different ($P < 0.05$).
[1]Each value represents the mean of 30 replicates with 6 birds for each grind size.
[2]Each value represents the mean of 12 replicates with 6 birds for each conditioning–pelleting temperature.
[3] SEM–pooled standard error of the mean (n = 60).

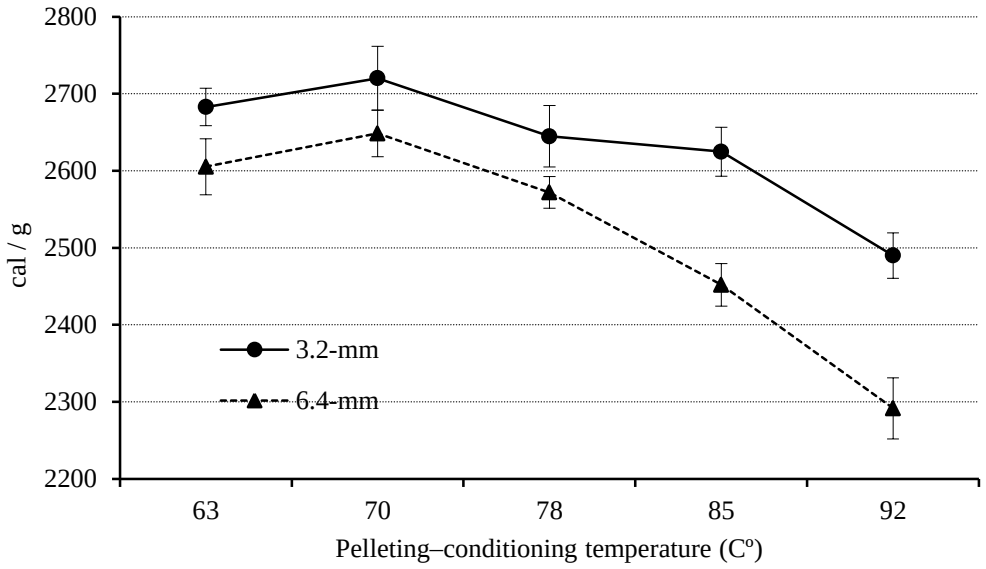

FIGURE 4.1. Effect of hammer–mill screen–hole size (3.2– and 6.4–mm) and pelleting–conditioning temperature (°C) on AMEn of pea–based diets fed to broilers (21 d). Each data point is the mean of 6 observations. Bars represent SEM and an asterisk (*) indicates sections for which a significant ($P \leq 0.05$) difference was found between pelleting–conditioning temperature.

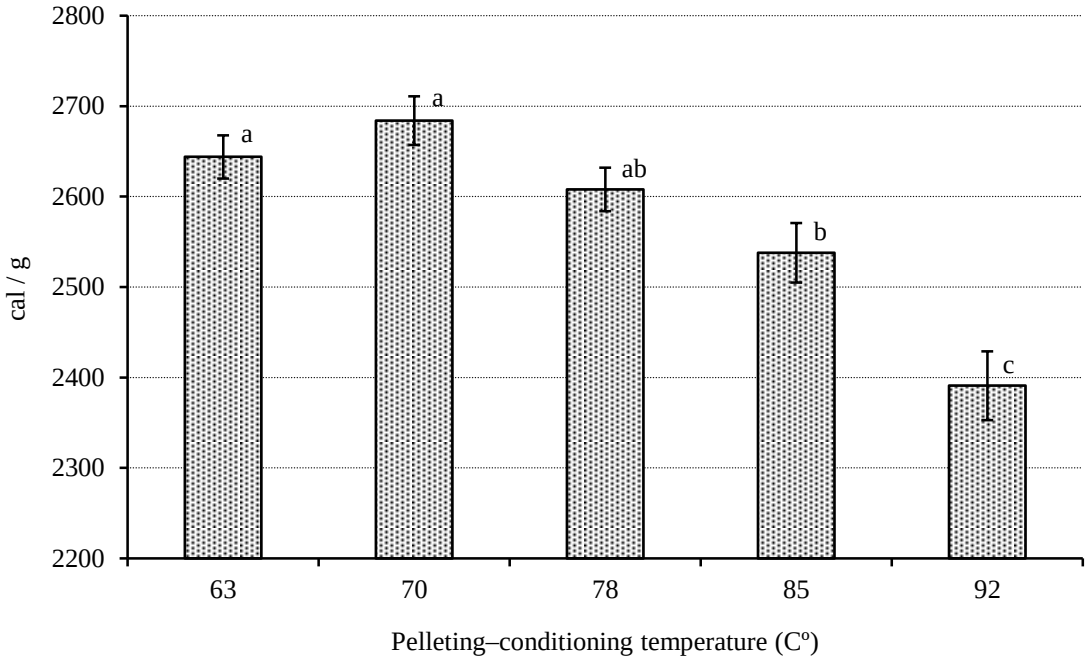

FIGURE 4.2. Effect of pelleting–conditioning temperature (°C) on AMEn of pea–based diets fed to broilers (21 d). Each data point is the mean of 12 observations (cages) each had 6 birds. Bars represent SEM. [a–c] Column not sharing a common superscript is different (*P* < 0.05).

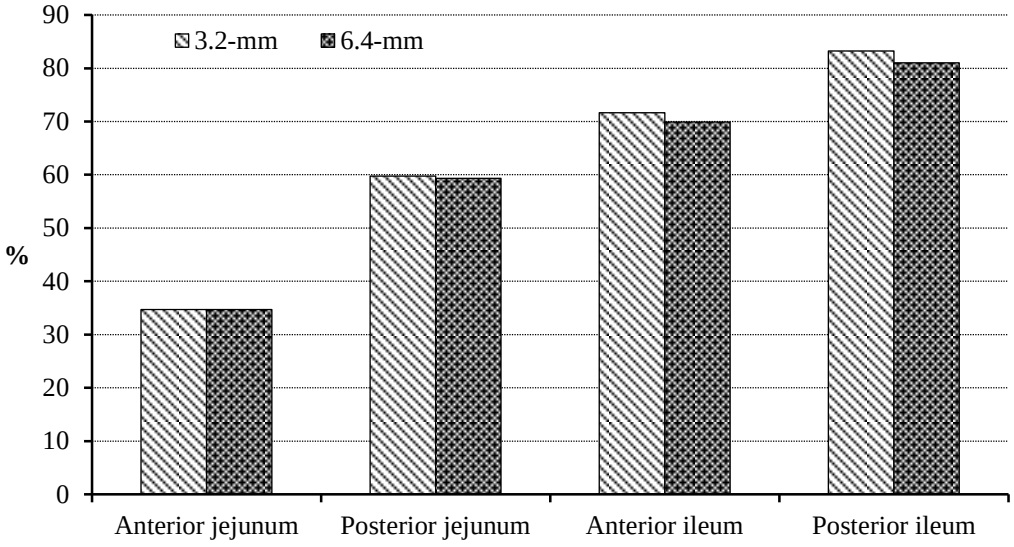

FIGURE 4.3. Effect of hammer–mill screen–hole size (3.2 and 6.4 mm) on starch digestion of pea fed to broilers (21 d). Each data point is the mean of 30 observations.

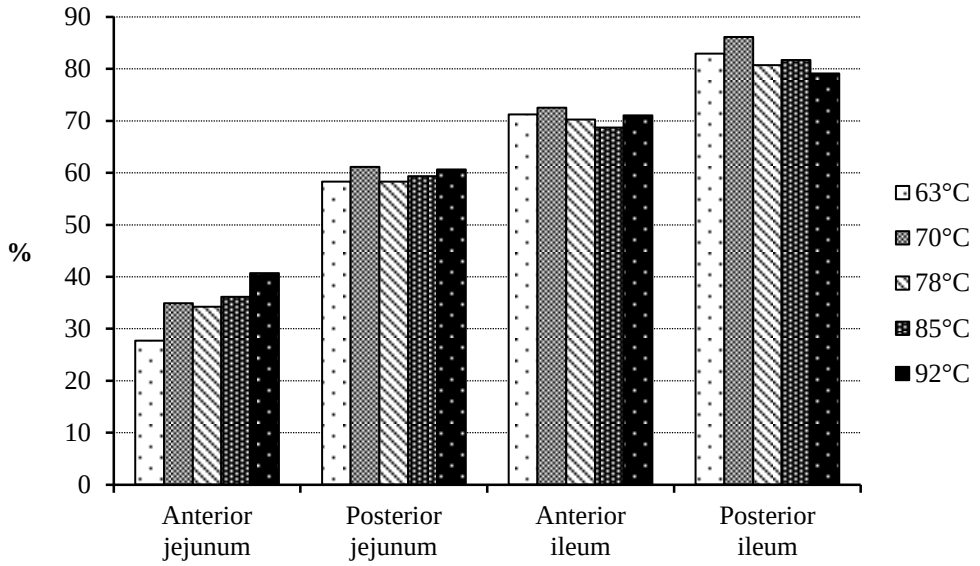

FIGURE 4.4. Effect of pelleting–conditioning temperature (°C) on starch digestion of pea fed to broilers (21 d). Each data point is the mean of 12 observations.

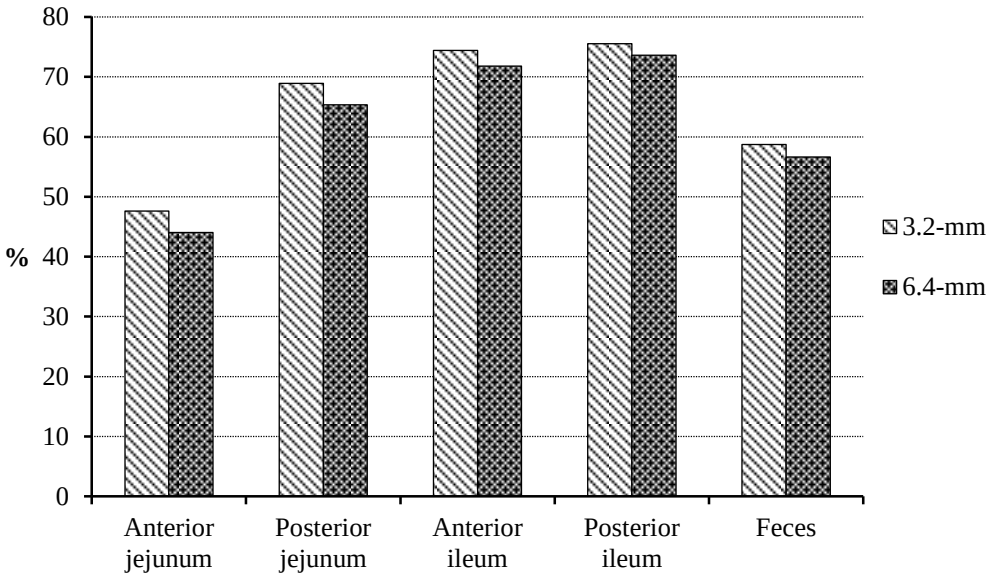

FIGURE 4.5. Effect of hammer–mill screen–hole size (3.2 and 6.4 mm) on protein digestion of pea fed to broilers (21 d). Each data point is the mean of 30 observations.

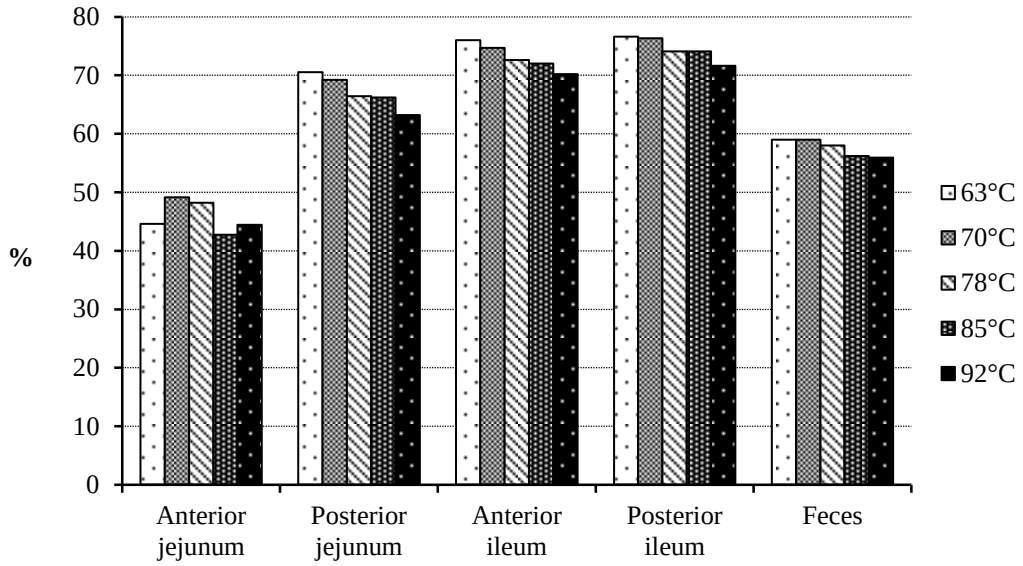

FIGURE 4.6. Effect of pelleting–conditioning temperature (°C) on protein digestion of pea fed to broilers (21 d). Each data point is the mean of 12 observations.

EFFECTS OF FEED PROCESSING AND CULTIVAR ON PEA NUTRIENT DIGESTIBILITY FOR POULTRY

5.0. IN VITRO PREDICTION OF STARCH DIGESTION OF PEA CULTIVARS AND CEREAL GRAINS AS AFFECTED BY SIEVE–HOLE SIZE AND PEA CULTIVAR

5.1. Abstract

Starch is the largest contributor of dietary energy in poultry diets. In vitro methods can be used to estimate the rate and extent of starch digestion in poultry feedstuffs. An in vitro assay procedure mimicking the gastric and small intestine conditions of chickens was established. The rate and extent of starch digestion for nine pea cultivars (Alfetta, Eclipse, CDC Minuet, CDC Montero, CDC Mozart, Nitouche, SW Salute, and CDC Striker) and three cereal grains (barley, corn, and wheat) and their responses to grinding (0.5–, 1.0–, and 2.0–mm sieve–hole sizes) were estimated in two experiments. Samples of pea cultivars grown in three consecutive years were used in experiment I. Samples of barley, corn, and wheat and one pea cultivar (Eclipse) were used in experiment II. Aliquots were taken at timed intervals (15, 30, 45, 60, 120, 180, 240, 300, and 300 min) of the small intestinal phase and glucose was measured colorimetrically using the glucose oxidase method. Starch digestibility was calculated based on total starch in the original grain sample. There was no interaction between pea cultivar or cereal grain and sieve–hole size. Pea cultivar affected both the rate and extent of starch digestion. Finer grinding resulted in more rapid and extensive starch digestion than coarse grinding. Wheat starch was rapidly digested followed by barley then corn and finally pea starch. In conclusion, this in vitro model confirmed the slowly digesting nature of pea starch in comparison to barley, corn, and wheat and demonstrated that pea cultivar and grind sieve–hole size affect the rate and extent of starch digestion.

Key words: starch, in vitro, particle size, pea, cultivar

5.2. Introduction

Feedstuffs are primarily evaluated on the basis of nutrient digestibility and these values are used in feed formulation. Dietary energy and protein are the key nutrients that are first considered in poultry diets. Starch supplies more than 50% of the metabolizable energy (**ME**) in poultry feed and it is well documented that the digestibility of starch has a significant impact on ingredient ME (Wiseman, 2000). Starch is not completely digested in the small intestine of monogastric species, including the chicken (Longstaff and McNab, 1987; Yutste et al., 1991). The rate and extent of starch digestion varies among starch sources (Weurding et al., 2001b). In vivo starch digestibility can be measured with a reasonable degree of accuracy, but the procedure is expensive, and therefore is less likely to be used for large numbers of samples or to compare cultivars in a statistically meaningful manner. In vitro assays are a timely and relatively inexpensive alternative method of assessing both the rate and extent of starch digestion in animal feedstuffs.

The nutritional value of starch is strongly related to its digestibility, which depends on its structure and processing. Starch digestion by amylolytic enzymes in the small intestine is affected by a number of factors including the size and shape of starch granules, amylose/amylopectin ratio, and crystalline structure (A, B, and C), as well as protein and lipid associations and cell walls that encapsulate them (Moran, 1982; Colonna et al., 1992; Classen, 1996; Oates, 1997; Tester et al., 2006; Lehmann and

Robin, 2007; Singh et al., 2010). The rate of starch digestion in the small intestine of broiler chickens also differs among starch sources (Weurding et al., 2001b). Moreover, the extent of starch digestibility is affected by the rate of starch digestion and the enzymatically resistant starch fraction (Carré, 2004). Starch digestibility of different feedstuffs can vary based on its botanical origin, which determines the physicochemical structure of starch (Tester et al., 2004; Singh et al., 2010).

Field pea starch is characterized by granule size ranging between 10 – 40 µm, a smooth granule surface, a high amylose/amylopectin ratio, approximately 8.1% of amylose–lipid complexes (Ratnayake et al., 2002), and C–type of crystalline structure. Such properties of pea starch may affect starch digestibility and slow the rate of digestion for pea starch compared with other cereal grains (Yutste et al., 1991; Weurding et al., 2001a).

Grinding is the most common method of feed processing and involves reduction in particle size. The nutritional value of pea is therefore influenced by the size of particles obtained after grinding (Carré et al., 1998). Grinding improves starch digestibility by increasing the surface area of starch granules and disrupting the cell wall therefore offering a greater accessibility for digestive enzymes (Behnke, 1996). Although differences in nutrient digestibility in field pea varieties have been reported (Carré et al., 1998; Gabriel et al., 2008), the effects of genetic origin of pea cultivar on the response to grinding have not been well investigated.

An in vitro method for assessing starch digestibility that mimicked the conditions of the human small intestine was first proposed by Englyst et al. (1992). Based on that method, starch from different sources can be classified as rapid digested (**RDS**), slow

digested (**SDS**), and indigestible (resistant starch – **RS**). Furthermore, RS can be sub–classified into physically inaccessible, resistant starch granule, and retrograded amylose. It is probable that digestible starch in chickens can be predicted by using an in vitro method, but the accuracy would be enhanced by using a model that more closely simulates the digestive conditions and processes of their digestive tract.

Even though in vivo digestion could never be precisely simulated by an in vitro procedure, the former is more expensive and time consuming. A consequence is that assessment of cultivar variation in starch digestion is usually not completed. Often research on cultivar variation in digestibility involves one sample per cultivar, an experimental design that is not statistically valid. A further advantage of an in vitro assay is the detailed ability to predict in vivo starch digestion. Therefore, a quick, reliable, and inexpensive laboratory method for determining the effect of pea cultivar and sieve–hole size on starch digestibility in feedstuffs would have value.

It was hypothesized that pea cultivar and sieve–hole size affects the in vitro rate and extent of starch digestibility and that cultivar by sieve–hole size interactions exist. Furthermore, it was hypothesized that pea starch is more slowly digested than starch from barley, corn, and wheat regardless of cultivar and sieve–hole size. Therefore, the objective of this research was to use the in vitro starch digestion procedure of Englyst et al. (1992) and modified it to more closely match the chickens digestive tract to study the effect of pea cultivar and sieve–hole size on the rate and the extent of starch digestibility, and compare the kinetics of pea starch digestion with other grains (barley, corn, and wheat).

5.3. Materials and Methods

Two studies were completed to measure the rate and extent of starch digestion using an in vitro model simulating the chicken digestive tract. The first experiment used samples of nine pea cultivars grown under the same conditions in three consecutive years and supplied by the Crop Development Centre (**CDC**), University of Saskatchewan. Samples were derived from field trials grown in Saskatchewan in 2004, 2005, and 2006. Field trials were managed under typical conditions for field pea production in Saskatchewan. Pea cultivars, namely DS Admiral, Alfetta, Eclipse, CDC Minuet, CDC Montero, CDC Mozart, Nitouche, SW Salute, and CDC Striker were evaluated in this experiment. CDC Montero, Nitouche, and CDC Striker are green–cotyledon cultivars, whereas the remaining cultivars have yellow cotyledons. The second experiment compared the in vitro starch digestion of a pea sample (Eclipse) with barley, corn, and wheat of unknown cultivar and origin.

5.3.1. Principle of the Method

Two incubation periods were established in order to mimic the conditions in the chicken's proventriculus/gizzard (gastric phase) and small intestine (SI phase). After the gastric phase, released glucose was measured colorimetrically using the glucose oxidase method at different incubation times in the small intestine phase. Based on released glucose, the starch content of the incubation sample was calculated and starch digestibility was estimated based on total starch value for the pea cultivar and grain samples.

5.3.2. Assay Procedure

The gastric phase was adapted from the method described by Bedford and Classen (1993) and the second incubation was a modified version of the in vitro procedure of Englyst et al. (1992). Modifications included using an incubation temperature of 41°C instead of 37°C and buffer pH was adjusted to 5.6 instead of 5.2. Aliquots for analysis were taken every 15 min during the first 60 min of the SI phase and then every 60 min up to a total of 360 min. The aliquots during the first 60 min were considered to represent the digestion of starch in the proximal part of the jejunum. Enzyme concentration during the SI phase was based on a pilot trial and neither total starch in grains nor digestion samples were corrected for free glucose, which is in contrast to the Englyst et al. (1992) method.

Enzyme solution I was prepared by adding 1.818 g of pepsin (EC 3.4.23.1; 66 U/mg solid; Sigma ref. P–7125; St. Louis, MO. USA) into 60 mL of 0.1 M of hydrochloric acid. The mix was stirred magnetically for 10 min and provided 2000 U/mL of pepsin and a pH of 2.5. For each in vitro trial, this solution was freshly prepared.

For enzyme solution II, 3.0 g of pancreatin (Sigma ref. P–7545; St. Louis, MO. USA) was added to each of nine centrifuge tubes (50 mL) and then 20 mL of distilled water was added to each tube. Then a stirrer was added and content was stirred magnetically for 10 min. Tubes were then centrifuged for 10 min at 1500 g (3000 rpm). From each tube a 14 mL supernatant was taken into a beaker (total amount of solution was 126 mL). At that point, 22.5 mL of amyloglucosidase (EC 3.2.1.3; Megazyme, Bray Business Park, Bray, Ireland.) and 9.0 mL of invertase (EC 3.2.1.26; Megazyme, Bray Business Park, Bray, Ireland.) were added to the beaker in order to provide 28.5 U/mL of

amyloglucosidase and 60 U/mL of invertase. The solution was prepared and mixed immediately before use.

To provide saturated benzoic acid solution for buffer preparation, 2.9 g of benzoic acid ($C_7H_6O_2$; Sigma ref. B–3250; St. Louis, MO. USA) was dissolved into 1.0 L of distilled water and then it was divided into 4 portions of 250 mL. Sodium acetate buffer was made by dissolving 13.6 g of sodium acetate trihydrate, ($CH_3COONa.3H_2O$; Sigma ref. S–6770; BDH ACS759; St. Louis, MO. USA) in 250 mL of saturated benzoic acid solution and pH was adjusted to 5.6 using acetic acid (0.1 M). The solution was then increased to 1.0 L with distilled water. Calcium chloride solution (1.0 M) was made by dissolving 11.1 g of $CaCl_2$ (Sigma ref. C–1016; St. Louis, MO. USA) with 100 mL of distilled water and 4 mL per liter were added to the buffer solution in order to stabilize enzyme activity. Ethanol solution 66% was prepared by mixing 2.81 of ethyl alcohol (95 %) in 4.01 L of distilled water. This solution was used to stop the enzyme activity in aliquots taken during the SI phase of the in vitro procedure.

5.3.3. In vitro Starch Degradation

Samples were ground in a centrifugal laboratory mill (Retsch Mill ZM1, Newtown, PA, USA) using 0.5–, 1.0–, or 2.0–mm sieve–hole sizes. Dry matter was determined at the same time as the in vitro analysis. All in vitro analyses were done in triplicate and the glucose oxidase method described below was done in duplicate. Two samples of corn starch and a blank were included as controls in each in vitro run. The blank tube was included to correct for the glucose content in the amyloglucosidase. All enzyme, buffer, ethanol, and GOPOD solutions were equilibrated to incubation temperature before being added.

5.3.3.1. First Incubation – Gastric Phase

For each test, between 700 to 900 mg of each sample and 50 mg of guar gum powder were weighed to the nearest 0.1 mg and added to polypropylene centrifuge tubes (50 mL with screw cap). Guar gum was used to standardize the viscosity of the mixture. Then 2.0 mL of prepared enzyme solution I was added into each sample. Tubes were stirred carefully on a vortex mixer, immediately capped and immersed horizontally in a shaking water bath (41°C) for 30 min. The shaking bath provided ±150 strokes/min and the length of stroke was ±30 mm. This incubation period was designed to simulate the conditions in the proventriculus and gizzard (gastric phase) and allow hydrolysis of protein by pepsin. After 30 min, tubes were removed from the water bath and 20 mL sodium acetate buffer was added to each tube. Samples and standards were mixed thoroughly on a vortex mixer. Buffer solution was equilibrated to 41°C before it was used.

5.3.3.2. Second Incubation – SI Phase

Three glass balls (1.5 cm ∅) and 5.0 mL of freshly prepared enzyme solution II were added to each tube. Tubes were capped, carefully mixed on vortex mixer, secured horizontally in the shacking water bath (41°C) and timing was started. Aliquots of 0.5 mL were carefully removed and placed into prepared labeled tubes (polypropylene centrifuge tubes, 50mL with screw cap) containing 20 mL of 66% ethanol. Aliquots were immediately mixed well on a vortex mixer. For all in vitro assays, aliquots were taken at 15, 30, 45, 60, 120,180, 240, 300, and 360 min after the initiation of the second incubation. Time required to add the enzyme solution II at the beginning of the in vitro

analysis and take the aliquots throughout the in vitro procedure was approximately 0.2 min per sample. Shaking of tubes was not stopped when taking aliquots.

Ethanol tubes with aliquots were centrifuged at 1500g for 2 min in order to obtain a clear supernatant. The amount of released glucose in each incubation sample was measured using a glucose oxidase method (Glucose oxidase diagnostic kit, K–GLC 10/05, Megazyme, Ireland). In summary, duplicate aliquots of 0.1 mL were placed into a labeled set of glass test tubes (round bottomed) and 3.0 mL of prepared GOPOD Reagent–buffer was added. Tubes were then incubated in a 50°C water bath for 30 min. A set of 4 tubes with 0.1 mL of glucose was included for standard and two tubes with 0.1 mL aliquots of distilled water were also included as a blank. The tubes were then taken out and left to equilibrate with room temperature. Tube content was transferred into labeled cuvettes (UV 4.5 μL PMMA) and assessed colorimetrically at 510 nm using a spectrophotometer (Milton Roy, Spectronic 601, USA). Glucose was determined as follows for all samples:

$$\text{Glucose (\%)} = [A_t \times V_t \times C \times D / A_s \times W_t] \times 100$$

Where A_t is the absorption of test solution, V_t is total volume of test solution, which sub-sample taken for glucose determination (26.5 + ml/g sample weight), C concentration of glucose mg/ml of standard (1.0 mg/ml), A_s is the absorbance of standard, W_t is the weight of sample in mg, D is the dilution factor (41). Starch was calculated by multiplying glucose % by 0.9.

5.3.4. Total Starch

All test samples were assayed for total starch. Samples were ground using a 0.2 mm sieve holes size in a centrifugal laboratory mill. Total starch was measured in

accordance to the AOAC (1990) Method 996.11, using the Megazyme kit for total starch assay (amyloglucosidase/α–amylase method, K–TSTA 01/05, Megazyme International Ireland Ltd., Bray, Co. Wicklow, Ireland). Released glucose was quantified colorimetrically using glucose oxidase method and starch was calculated:

$$TS\ (\%) = [A_s \times (0.1 \div A_g) \times 1000] \div W \times 0.9 \times 100$$

where A_s is the average absorbance of a sample read against the reagent blank, A_g = 0.1(mg glucose)/absorbance for 0.1 mg glucose standard, 1000 is the volume correction (0.1 ml taken from 100 ml), W is the weight in mg of analysed sample, 0.9 is the correction factor from free glucose to anhydrous glucose (starch), 100 is the factor that allows to expresses starch as a percentage of sample weight.

5.3.5. Starch Digestibility

Starch digestibility was calculated using the following formula:

$$\text{Starch digestibility}\ (\%) = (TS_{\text{in vitro}} \div TS) \times 100$$

where $TS_{\text{in vitro}}$ is the total starch at a specific interval of the incubation and TS is the total starch of the sample.

5.4. Statistical Analysis

Data were analyzed as two experiments. The first experiment examined the main effects of nine pea cultivars and 3 sieve–hole sizes and their interaction. The second experiment compared a pea cultivar (Eclipse) to barley, corn, and wheat after grinding to various levels of fineness. The first experiment utilized a Randomized Complete Block Design (RCBD) analyzed as a two–way factorial (9 × 3) arranged with the main effects of nine pea cultivars and three grind sizes (0.5–, 1.0– and 2.0–mm of sieve–hole size). Data were blocked by year (3 years) and there were 3 replicates per treatment within each

block. The second experiment was conducted as a Complete Randomized Design (CRD) and analyzed as a two–way factorial. The main effects consisted of grains (barley, corn, wheat, and pea) and two grind sizes (0.5– and 1.0–mm of sieve–hole size). In this experiment, there were 3 replicates per treatment. The normality of the data was checked prior to analysis using the Shapiro–Wilk. All data were subjected to analysis of variance using the PROC Mixed procedure of SAS Institute (2008). When ANOVA indicated a significant treatments effect, Tukey's Studentized Range Test was used for mean separation and pdmix800 was used to provide letters for differences (Saxton, 1998). Differences were considered significant when the probability of difference was less than or equal to 0.05 unless otherwise stated. Because block (year) was not significant, it was removed from the statistical model and data for pea cultivar from the three years were combined.

5.5. Results

5.5.1. Experiment 1. Effects of Pea Cultivar and Sieve–Hole Size on the Kinetics of Pea Starch Digestion

Total starch and crude protein of pea cultivars are presented in Table 5.1. Among the studied pea cultivars from three years, total starch varied between 421 to 508 g/kg (DM) and crude protein ranged between 214 to 262 g/kg (DM). There were no differences between studied years (blocks) on starch digestibility of pea cultivars, and therefore data from the three years were combined. There were no interactions between pea cultivar and sieve–hole size on digestibility of pea starch, hence the main effects of cultivar and sieve–hole size are discussed separately.

5.5.1.1. Pea Cultivar

The effect of pea genotype was evaluated in nine cultivars. The results of the in vitro starch digestibility of pea cultivars are given in Table 5.2. The rate and extent of starch digestibility of pea were affected by pea cultivar. Starches from pea cultivars varied in digestibility from low of 3.4% at 15 min incubation to a high of 9.2% at 180 min incubation. The curves of in vitro digestion of overall pea cultivars show a higher rate of starch degradation in the initial stage up to 120 min of incubation and a flattening trend towards 360 min of incubation (Figure 5.1). After 60 min of starch digestion, released glucose increased rapidly in the aliquots and almost half of pea starch was converted to glucose, however only 19% additional starch was digested at 120 min and finally the rate of starch degradation slowed down to 11, 6, 5, and 4% increases in starch digestibility for the following incubation times of 180, 240, 300, and 360 min, respectively. Almost 50% of pea starch was hydrolyzed rapidly within 120 min and less than 40% was digested slowly after 120 min and up to 360 min of incubation.

It is interesting to note that the starch digestion was comparatively rapid initially for some of the cultivars but the digestion slowed down later. For instance, Eclipse had rapid starch digestion within 120 min of in vitro incubation while it slowed down after 180 min. In contrast, CDC Minuet, Nitouche, and CDC Striker had the opposite digestibility trend. The rate of starch digestibility was constantly lower for SW Salute and DS Admiral cultivars compared to rest of the cultivars during all the incubation times. In contrast, the rate of starch digestion was higher for Eclipse cultivar than that for the other cultivars studied during the first 180 min. The extent of starch digestion of different pea

cultivars varied from a low of 83.7% (SW Salute) to a high 91.5% (Alfetta) after 360 min of incubation.

5.5.1.2. The Effect of Sieve–Hole Size on the Kinetics of Pea Starch

The effect of using fine grind size on the in vitro digestion of pea starch was also investigated. Fine grinding increased the rate and the extent of the in vitro starch digestibility for all pea cultivars (Table 5.3). After 30 min of incubation the starch digestibility was significantly different among all sieve–hole sizes tested. Finer grind size resulted in more rapid starch digestion; for example, after 120 min of incubation starch digestion was 53.4, 63.4, and 71.0% for 2.0–, 1.0– and 0.5–mm sieve–hole size, respectively. Moreover, after 360 min of incubation, the extent of pea starch digestibility was 96.3% for 0.5 mm sieve–hole size in contrast to 90.4 and only 77.8% for 1.0– and 2.0–mm sieve–hole size, respectively.

5.5.2. Experiment 2. The Kinetics of Starch Digestion of Pea vs Grains

The kinetics of starch digestion of barley, corn, wheat, and pea was investigated in a separate experiment. The results of this in vitro assay are presented in Table 5.4. After 60 min, starch digestion was 91.5, 85.5, 72.7, and only 43.7% for wheat, barley, corn, and pea; respectively. The rate of starch digestibility was significantly different among wheat, barley, corn, and pea (Eclipse) until 120 min of incubation with wheat having the highest rate of digestibility and pea the lowest (Figure 5.5). Starch from wheat was digested most rapidly, followed by barley, corn, and finally pea starch. Within the first 120 min, the rate of starch digestion was significantly different among wheat, barley, corn and pea. Only 65.8% of pea starch was digested after 120 min of incubation compared to 98.6, 94.6, and 89.6% of wheat, barley, and corn; respectively. It is

interesting that neither the rate nor the extent of digestion for cereal grain starches were affected by the size of sieve–hole (Table 5.5). In contrast Eclipse pea starch in Experiment 2 had the same behavior as that found in Experiment 1, where size of sieve hole was 1.0 mm.

5.6. Discussion

The optimum utilization of a feedstuff is influenced by the degree of nutrient digestibility and in the case of energy the extent of starch digestion has a pronounced effect (Carré et al., 1998). Moreover, it has been reported that the rate of starch digestion may also affect bird productivity (Weurding et al., 2003; Gutierrez del Alamo et al., 2009). Pea starch digestibility can vary widely depending on cultivar or the nature of processing. Therefore, to optimize the utilization of pea in practical poultry feed, it is important to characterize these factors. However, because of the cost, time, and the limitation of screening a large number of samples using an in vivo method of starch digestibility, in vitro methods are an attractive alternative. An in vitro procedure, based on a modified version of the method described by Englyst et al. (1992), was developed to study the kinetics of starch degradation as affected by pea cultivar, grain source (barley, corn, and wheat), and sieve–hole size.

In Experiment 1, no interactions were found between pea cultivar and sieve–hole size for any of the time points studied and the lack of interaction indicates that all cultivars are responding in the same way to the effect of sieve–hole size. This further suggests that the reasons for slower starch digestion are inherently the same in all cultivars.

Weurding et al., (2001a) found significant correlations between in vitro starch degradation at 120 and 240 min and in vivo starch digestion by the end of posterior jejunum and posterior ileum, respectively. Based on these correlations, starch from different pea cultivars can be classified based on digestion at 120 and 240 min of the small intestinal phase of in vitro incubation. The fraction of starch digested within 120 min of incubation can be defined as RDS whereas the starch portion digested after 120 min can be defined as SDS. The starch fraction that is not digested after 240 min of incubation is defined as RS. These definitions reflect the shorter digestion period in the gastrointestinal tract of chickens and are different from those originally introduced for humans by Englyst et al., (1992). In their definitions, starch that was digested within the first 120 min is classified as a RDS, whereas the SDS is defined as starch that was digested between 120 min and 360 min of incubation. Starch not hydrolyzed by 360 min was categorized as a RS.

In this experiment, starch and protein content of studied pea cultivars varied (Table 5.1) and this finding is in agreement with Hood–Niefer et al. (2011). The results of the in vitro study using 9 pea cultivars demonstrated that pea cultivar affected both the rate and extent of starch digestion, but starch from all cultivars would still be considered to have a high proportion of SDS. The reason for the significant cultivar effects can't be assessed based on this research, but differences in physiochemical structure of the starch may be responsible (Carré, 2004; Tester et al., 2004). Factors such as encapsulation by protein matrixes and cell walls (Longstaff and McNab, 1987) may slow down or reduce starch digestion as can other starch characteristics such as amylose/amylopectin ratio and crystalline structure (Oates, 1997; Lehmann and Robin, 2007; Singh et al., 2010). The

results of an in vitro study showed a significant reduction in rate of starch degradation in high amylose barley compared to samples with low or normal amylose levels (Stevnebø et al., 2006). The amylose content in pea starch can be variable (33.1 to 49.6% according to Ratnayake et al. (2002) and it would have been of interest to have characterized the pea cultivars used in this research to determine if this variation is responsible for the cultivar starch digestion effects. It can be concluded that the variation in rate and extent of pea starch digestion is based on the genetic differences between these cultivars and these differences are large enough to warrant further investigation. The fraction of undigested starch can be classified as RS and the impact of this fraction is likely less significant in avian than in mammalian species because of the reduced fermentation capacity in the post–ileal digestive tract.

Feedstuffs are routinely processed in poultry diets and grinding (particle size reduction) is usually applied to reduce feed wastage, increase feed consumption, and ultimately improve feed efficiency. In this study, the 1.0–mm sieve–hole size was used to simulate the grinding action in the gizzard of chickens whereas 0.5– and 2.0–mm were used to represent the fine and coarse sizes, respectively. As expected, the application of the small sieve–hole size resulted in a greater degree of starch degradation (Table 5.3), which is a consequence of increased particle surface area and increased digestive enzyme accessibility (Colonna et al., 1992; Tester et al., 2006). Finer grinding also reduces the impact of the physical barriers that protect the starch granule from enzyme attack (Carré, 2004). The extent of starch digestion at 360 min of the in vitro degradation of pea samples was 96.3, 90.4, and 77.8% for samples ground using 0.5, 1.0 and 2.0 mm sieve–holes size, respectively. The variation in pea starch digestibility results from interactions

between a reduced accessibility of digestive enzymes in coarse particles and the resistant structure of starch granules. The lack of interaction between sieve–holes size and pea cultivar on the rate and extent of starch digestibility of all pea cultivars shows the parallel nature effect of fineness on starch degradation regardless the pea genotype.

In Experiment 2, the kinetics of starch degradation of a pea cultivar (Eclipse) and cereal grains (barley, corn, and wheat) were compared. These feedstuffs vary in both starch characteristics and also in starch granule structure. The physiochemical structure of starch granule itself, cell walls, and protein matrix are most likely affect digestion of starch (Wiseman et al., 2000; Tester et al., 2004). The slow digestion rate and low extent of starch digestion of pea agrees with earlier findings (Yutste et al., 1991) and are supportive of other research (Weurding et al., 2001b). It is documented that legume starches are less digested than cereal grains (Hoover and Zhou, 2003) and the low digestibility of legume starches can be attributed to a number of factors including encapsulation of starch, a high level of amylose, and C–type crystallinity (Lehmann and Robin, 2007). Moreover, starch digestion may be slowed by the physical structure of pea starch granules such as the cell walls and protein matrix (McAllister et al., 1993). Similarly, others have found that a considerable portion of pea starch is not digested by the end of ileum compared to wheat– or corn–starch (Longstaff and McNab, 1987; Daveby et al., 1998; Weurding et al., 2001b; Meng and Slominski, 2005).

The three grains were also different in starch digestion rate with a digestion ranking of wheat > barley > corn up to 120 min of digestion. Although the values are quite high and differences small by this time, they still demonstrate differences in the proportion of RDS in these samples. At 240 min, wheat and barley starch digestibility

values are equal and higher than corn by approximately the same amount as at 120 min. This suggests that in addition to differences in RDS, these samples also differed in the proportion of RS. In contrast to Weurding et al. (2001a), the corn sample was digested more slowly and not to the same extent as wheat in vitro. Although corn is generally considered to be highly digestible by chickens, low starch digestibility has been reported (Noy and Sklan, 1995). Fischer (2003) compared feeding wheat with and without dietary enzymes to feeding a corn diet, and found that propionic acid production in caeca was higher for the corn fed birds. She speculated that this was due to lower starch digestion for corn. Supporting this suggestion is research with cattle showing an increase in proprionic acid in the rumen of animals fed higher levels of starch (Van Soest, 1981; Pylot et al., 2000). Because only one sample of each grain sample was used in this study, it is not possible to say if the differences noted were due to sample or are true differences between grains. The finding that the starch digestibility of barley, corn, and wheat did not change with fineness of grind whereas the pea sample was affected is in general agreement with in vivo studies on the effect of processing on these seeds.

The in vitro model used in this research to determine the rate and the extent of starch digestion is a relatively quick, inexpensive, standardized, and repeatable method in comparison to in vivo studies on these nutritional characteristics; it is also preferred from an animal use standpoint. Although research is required to fully demonstrate the accuracy of this method in predicting in vivo starch digestibility, differences noted between grains are in agreement with previous research suggesting the model has value. As starch digestibility is positively correlated with ME of poultry diet (Wiseman, 2006; Weurding

et al., 2001a), using an in vitro method can help characterize and select feedstuffs that are to be used in poultry feeding.

In conclusion, pea cultivar affects both the rate and extent of starch digestion. The importance of these differences remains to be determined in comparisons of these data with in vivo experimentation. Grinding as the most widely used feed processing technique causes a major increase in pea starch digestibility. The variation between pea cultivars or samples and cereal grains as well the degree of particle size reduction due to grinding should be considered when pea is used as a feed ingredient for poultry. Finally, it is confirmed that pea starch is slowly digested compared to barley, corn, and wheat starch and based on previous research this might have a beneficial effect on poultry performance. The model used in this experiment offers a tool for plant breeders to select pea cultivars based on starch digestion that are suitable for poultry diets.

TABLE 5.1. Total starch and crude protein (g/kg dry matter) of pea cultivars

Pea cultivar	Crude protein (N × 6.25)			Total starch		
	2004	2005	2006	2004	2005	2006
DS Admiral	252	214	234	479	482	438
Alfetta	229	222	227	496	498	424
Eclipse	237	221	228	489	486	450
CDC Minuet	229	233	231	495	508	421
CDC Montero	226	223	219	491	484	443
CDC Mozart	239	235	229	502	496	452
Nitouche	262	256	241	455	446	433
SW Salute	240	224	237	474	478	440
CDC Striker	259	244	259	457	474	430
Range	226–262	214–256	219–259	455–502	446–508	421–452
Mean	241	230	233	481	484	437

TABLE 5.2. Effect of pea cultivar on in vitro starch digestibility[1] (%) at in minutes after the initiation of the small intestinal phase of the in vitro model

Pea cultivar	Incubation time (min)								
	15	30	45	60	120	180	240	300	360
DS Admiral	15.9^{bc}	26.1^{cd}	33.3^{cd}	40.4^{cd}	59.4^{de}	70.6^{c}	78.9^{ab}	83.1^{bcd}	87.4^{ab}
Alfetta	18.1^{a}	29.3^{abc}	37.2^{ab}	45.3^{ab}	64.8^{abc}	75.5^{ab}	81.6^{a}	86.9^{ab}	91.5^{a}
Eclipse	18.5^{a}	30.7^{a}	39.7^{a}	47.4^{a}	66.6^{a}	77.3^{a}	80.3^{a}	85.6^{abc}	86.6^{ab}
CDC Minuet	16.4^{abc}	26.6^{bcd}	34.7^{bcd}	42.6^{bc}	61.3^{dc}	72.0^{bc}	78.8^{ab}	84.5^{abc}	88.5^{ab}
CDC Montero	18.2^{a}	29.7^{ab}	37.7^{ab}	45.8^{ab}	62.7^{bcd}	72.9^{abc}	79.4^{ab}	84.2^{abc}	87.9^{ab}
CDC Mozart	17.5^{ab}	28.7^{abc}	37.4^{ab}	45.0^{ab}	62.3^{bcd}	73.2^{abc}	79.4^{ab}	82.6^{cd}	88.3^{ab}
Nitouche	17.5^{ab}	28.7^{abc}	36.8^{ab}	45.6^{ab}	65.5^{ab}	75.2^{ab}	83.2^{a}	87.7^{a}	89.8^{a}
SW Salute	15.1^{c}	24.0^{d}	32.3^{d}	38.8^{d}	57.4^{e}	69.0^{c}	74.7^{b}	79.8^{d}	83.7^{b}
CDC Striker	16.4^{abc}	27.6^{abc}	35.7^{bc}	43.8^{b}	63.3^{abc}	75.7^{ab}	82.3^{a}	87.0^{ab}	89.9^{a}
SEM[2]	0.48	0.74	0.71	0.73	0.81	1.02	1.23	0.95	1.23

[a-d] Means with different superscripts within a column are significantly different ($P \leq 0.05$).
[1] Values are proportion of digested starch at each interval of incubation time.
[2] SEM – pooled standard error of mean.
n = 27 samples.

TABLE 5.3. Effect of sieve–hole size on in vitro starch digestibility[1] (%) of pea in minutes after the initiation of the small intestinal phase of the in vitro model

Sieve–hole size	Incubation time (min)								
(mm)	15	30	45	60	120	180	240	300	360
0.5	18.7[a]	30.9[a]	41.9[a]	51.8[a]	71.0[a]	81.7[a]	88.6[a]	93.4[a]	96.3[a]
1.0	18.4[a]	31.2[a]	37.0[b]	44.2[b]	63.4[b]	75.8[b]	81.4[b]	86.1[b]	90.4[b]
2.0	14.1[b]	21.7[b]	29.3[c]	35.5[c]	53.4[c]	63.0[c]	69.6[c]	74.2[c]	77.8[c]
SEM	0.29	0.49	0.55	0.63	0.64	0.65	0.67	0.62	0.65

[a-c] Means with different superscripts within a column are significantly different ($P \leq 0.05$).
[1] Values are proportion of digested starch at each interval of incubation time.
[2] SEM–pooled standard error of the mean.
n = 81 samples.

TABLE 5.4. Effect of starch source on in vitro starch digestibility[1] (%) of barley, corn, wheat, and pea in minutes after the initiation of the small intestinal phase of the in vitro model

Grains	Incubation time (min)								
	15	30	45	60	120	180	240	300	360
Barley	31.7[b]	58.8[b]	79.8[b]	85.8[b]	94.6[b]	96.6[a]	97.8[a]	97.4[ab]	97.6[ab]
Corn	23.8[c]	45.2[c]	64.3[c]	72.6[c]	89.6[c]	89.8[b]	93.1[b]	93.0[b]	93.3[b]
Wheat	46.7[a]	75.9[a]	92.9[a]	91.5[a]	98.4[a]	97.7[a]	99.3[a]	99.2[a]	99.4[a]
Pea	15.7[d]	29.1[d]	36.2[d]	43.7[d]	65.8[d]	79.4[c]	86.6[c]	87.2[c]	89.1[c]
SEM[2]	1.08	1.17	1.33	1.30	0.87	1.12	0.94	1.17	0.71

[a-d] Means with different superscripts within a column are significantly different ($P \leq 0.05$).
[1] Values are proportion of digested starch at each interval of incubation time.
[2] SEM–pooled standard error of the mean.
n = 12 samples.

TABLE 5.5. Effect of starch source and sieve–hole size on in vitro starch digestibility[1] (%) of barley, corn, and wheat in minutes after the initiation of the small intestinal phase of the in vitro model

		Incubation time (min)								
Grains	Sieve–hole size (mm)	15	30	45	60	120	180	240	300	360
Barley	0.5	37.2	60.4	82.6	89.8	95.2	96.8	98.7	98.8	98.7
	1.0	26.3	57.2	76.9	81.8	94.1	96.4	97.9	97.8	97.4
Corn	0.5	39.0	44.8	61.2	71.6	87.2	89.2	94.3	94.8	94.4
	1.0	23.2	45.5	67.4	73.5	92.0	90.3	92.0	93.2	93.1
Wheat	0.5	52.7	77.7	91.4	94.1	97.8	98.1	99.7	99.8	99.5
	1.0	40.7	74.1	94.5	88.9	98.9	97.3	97.9	98.6	98.3
	SEM[2]	2.58	3.64	4.50	3.94	2.71	1.61	1.16	1.17	0.94

[a-d] Means with different superscripts within a column are significantly different ($P \leq 0.05$).
[1] Values are proportion of digested starch at each interval of incubation time.
[2] SEM–pooled standard error of the mean.
n = 6.

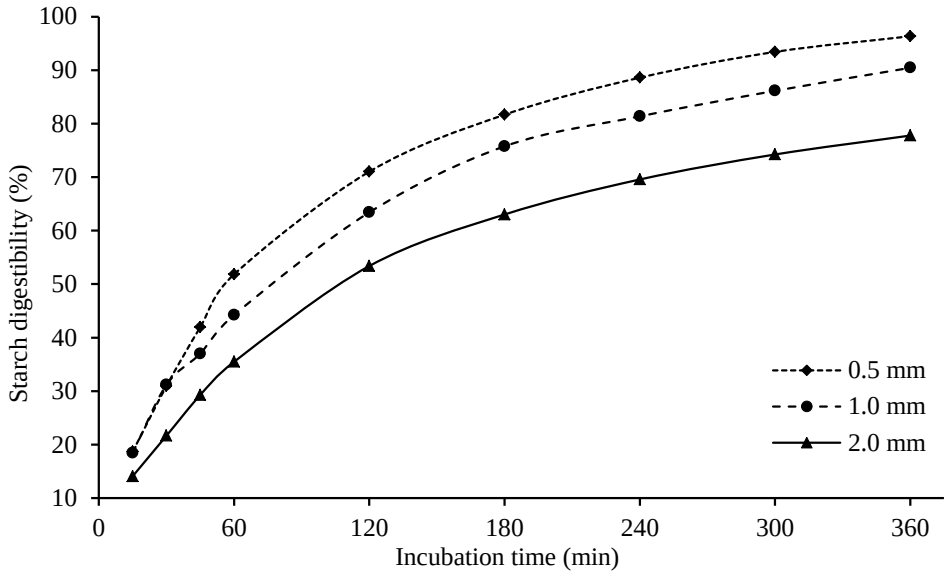

FIGURE 5.1. In vitro starch digestion for pea cultivars as affected by sieve–hole size in minutes after the initiation of the small intestinal phase of the in vitro model. Each point represents the mean and standard error of the mean of 81 aliquots.

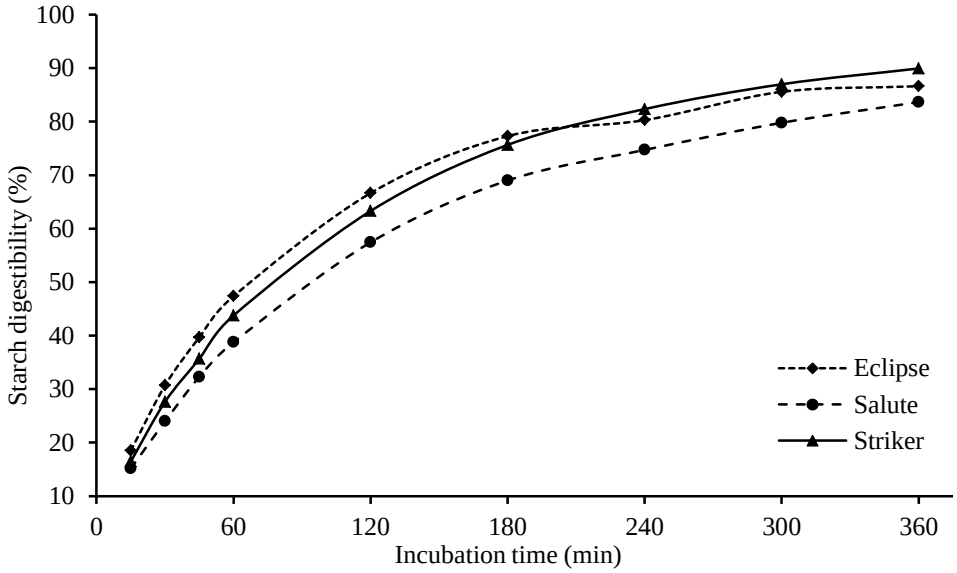

FIGURE 5.2. In vitro starch digestion for pea cultivars (Eclipse, SW Salute, and CDC Striker) in minutes after the initiation of the small intestinal phase of the in vitro model. Each point represents the mean of 27 aliquots.

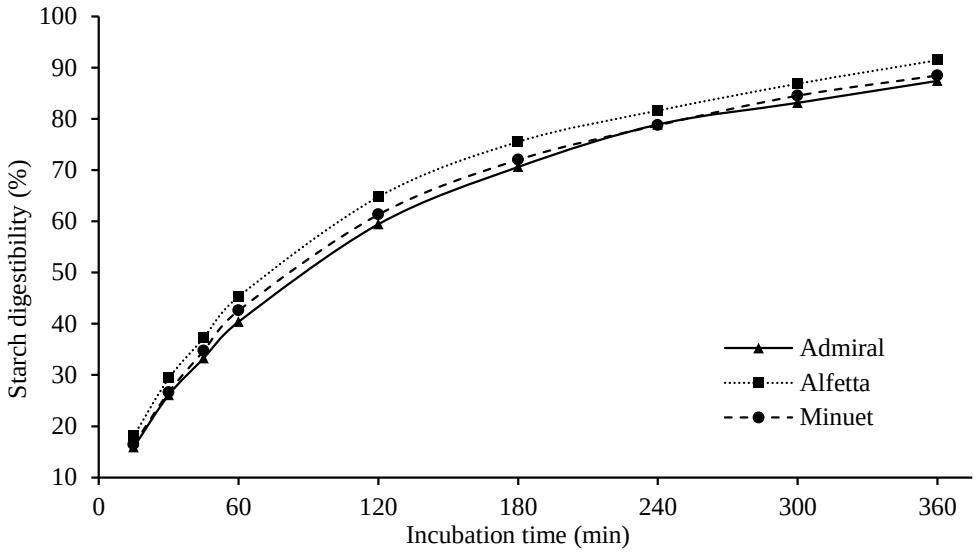

FIGURE 5.3. In vitro starch digestion for pea cultivars (DS Admiral, Alfetta, and CDC Minuet) in minutes after the initiation of the small intestinal phase of the in vitro model. Each point represents the mean of 27 aliquots.

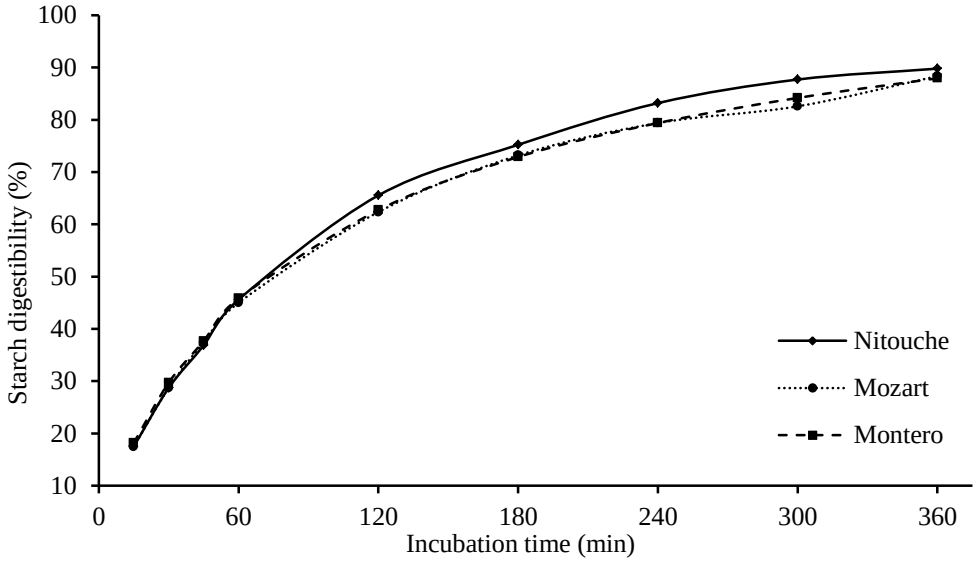

FIGURE 5.4. In vitro starch digestion for pea cultivars (CDC Montero, CDC Mozart, and Nitouche) in minutes after the initiation of the small intestinal phase of the

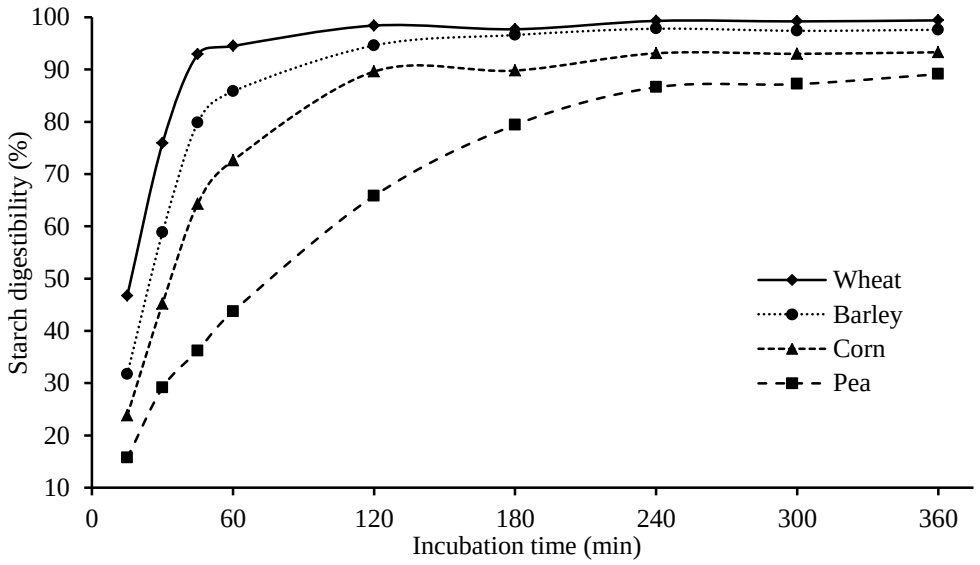

FIGURE 5.5. In vitro starch digestion for barley, corn, wheat, and pea in minutes after the initiation of the small intestinal phase of the in vitro model. Each point represents the mean of 12 aliquots.

6.0. NUTRIENT DIGESTIBILITY OF PEA CULTIVARS, BARLEY, CORN, AND WHEAT AS AFFECTED BY HAMMER–MILL SCREEN–HOLE SIZE AND PELLETING IN BROILER CHICKENS

6.1. Abstract

The nutritional value of pea (*Pisum sativum* L.) has been shown to be affected by processing to a larger degree than other energy contributing grains, but how this effect is influenced by pea cultivar has not been investigated. Therefore, the aim of this research was to study the impact of screen–hole size, feed form, and pea cultivar on dietary energy and protein digestibility. For comparison, single samples of cereal grains (barley, corn, wheat) were processed similarly. A 9 × 2 × 2 factorial arrangement was used to examine the effect of nine pea cultivars, two screen–hole sizes (3.2–, 4.6–mm), and feed form (mash, pellet), and a 3 × 2 × 2 factorial arrangement was used to examine the effect of three cereal grains (barley, corn, and wheat), two screen–hole size (3.2–, 4.6–mm), and feed form (mash, pellet). Pea–based diets were formulated to derive starch and crude protein solely from the pea cultivars. Cereal grain based diets were formulated to provide starch from grains. AME, AME_n, and apparent ileal protein digestibility (**AIPD**) were affected by pea cultivar. Fine grinding size (3.2–mm) and pelleted form improved energy value and protein digestibility. Cereal grains had minor responses to feed processing compared with pea. It was concluded that pea–based diets are more sensitive to feed processing than cereal grain based diets.

Key words: pea, screen–hole size, feed form, AME, protein digestibility

6.2. Introduction

Field pea (*Pisum sativum* L.) is characterized as having moderate levels of protein and energy, and therefore has value as a feedstuff in poultry diets (Gatel, 1994, Castell et al., 1996; Hickling, 2003; Wang and Daun, 2004). However, variability in published nutritional value, especially for poultry, may influence the recommended level of pea inclusion in the diets of broilers and other classes of poultry (Brenes et al., 1993; Castell et al., 1996; Igbasan and Guenter, 1996; Farrell et al., 1999; McNeill et al., 2004; Nalle et al., 2011). Potential factors contributing to variable results include pea cultivar and growing conditions, feed formulation and processing, and the methodology used to evaluate its nutritional value.

Levels of pea in poultry diets may also be limited by the presence of anti–nutritional factors (**ANFs**). ANFs such as amylase inhibitors, protease inhibitors, lectins, condensed tannins, and non–starch polysaccharides (**NSP**) may be found in variable amounts in pea (Gatel and Grosjean, 1990). As a result, low nutrient digestibility may occur and bird performance might be depressed. However, it has been documented that the amount of ANFs in pea is affected by pea cultivar and growing conditions. Moreover, new pea cultivars have been bred based on the low levels of ANFs. Indeed, spring–seeded pea cultivars grown in Western Canada contain less ANFs than winter–seeded pea (Gatel, 1994; Castel et al., 1996).

Pea seeds contain a considerable amount of starch, which is by far the greatest source of energy in poultry diets. However, susceptibility of pea starch granules to digestive enzymes is less than other conventional cereal grains (Longstaff and McNab, 1987; Weurding et al., 2001). Feed processing such as grinding and pelleting that alter

starch structure may increase starch granules accessibility to enzymatic hydrolysis and to some extent inactivate ANFs, and thereby improve the nutritional value of pea for poultry (Igbasan and Guenter, 1997).

In contrast to other grains, the apparent and true metabolizable energy (AME, TME) for pea have been determined in relatively few studies using broiler chickens and most frequently utilized only one pea sample (Moran et al., 1968; Carré et al. 1991; Igbasan and Guenter 1996a,b; Igbasan et al., 1997; Nalle et al., 2011). Furthermore, for the reasons outlined above, not all previous research may be predictive of the nutritional value of pea cultivars found in Western Canada and therefore not accurate for use in poultry feed formulation. Additional information on the nutritional value of pea for poultry, with specific emphasis on variation in Western Canadian cultivars, would increase the accuracy of feed formulation. Because of the important effect of feed processing on pea nutritional value, it is also relevant to study the effect of primary processing techniques (grinding, pelleting) on pea nutritional value and investigate the interaction between pea cultivar and processing. It is also of interest to compare the effect of processing on pea and cereal grains (barley, corn, and wheat) included in poultry diets.

It was hypothesized that pea cultivar would affect the ME and apparent protein digestibility of pea and the effect of feed processing on pea nutritional value would be more pronounced than on cereal grains. To test these hypotheses, one experiment evaluated the effect of pea cultivar, feed processing (screen–hole size and feed form) on the AME, AME_n, and apparent protein digestibility of pea fed to broiler chickens. A second experiment studied the effect of the same feed processing on the AME, AME_n, and apparent protein digestibility of barley, corn, and wheat.

6.3. Materials and Methods

The experimental protocol was approved by the Animal Care Committee of the University of Saskatchewan and experimental procedures were conducted in accordance with the Guide to the Care and Use of Experimental Animals, Canadian Council on Animal Care (1993).

6.3.1. Birds and Housing

To accommodate the number of experimental treatments and the limited number of battery cages, three consecutive trials were conducted with two replications of each treatment included in each trial. In each trial, a total of 576 one–day–old male broiler chicks (Ross × Ross 308) were obtained from a commercial hatchery (Lilydale Hatchery, Wynyard, SK. Canada) and housed in battery cages (50 cm width, 85 cm length, 25 cm high) with wire mesh floors. The cages were equipped with a trough feeder and two cup drinkers. The experimental room was environmentally controlled and temperature was initially set to 32°C on d 0 and gradually decreased 2.8°C per week during the experiment. The lighting program was 23L:1D with 30 to 40 lx during the first week and 20L:4D with 10 to 15 lx for the remainder of the experiment. Birds were provided ad libitum access to water and feed during the course of the experiment. On d 14 of age, birds were weighed on a cage basis (6 birds per cage), and cages were randomly assigned to one of the 48 dietary treatments (2 replications per treatment), and fed experimental diets.

6.3.2. Experimental Diets

A starter diet was formulated with nutrient levels meeting or exceeding the recommended values from Aviagen (2009) for male and straight–run broilers 1.8 kg. Pea, barley, corn, and wheat were ground using a 3.2 mm screen–hole size prior to diet mixing and pelleting (conditions explained later). The starter diet was fed from d 1 to 14 and feed was fed in a crumble form. The ingredient composition and calculated nutrient analysis of starter diet is presented in Table 6.1.

Experimental diets were formulated based on nutritional recommendations by Aviagen (2009). Nine pea diets were formulated using pea as the only source of carbohydrate and the main source of amino acids with DL–Methionine as the only additional source of amino acids. Pea cultivars, namely DS Admiral, Alfetta, Eclipse, CDC Minuet, CDC Montero, CDC Mozart, Nitouche, SW Salute, and CDC Striker were fed in this experiment. CDC Montero, Nitouche, and CDC Striker were green cotyledon cultivars, whereas the remaining cultivars had yellow cotyledons. The pea samples were provided by the Crop Development Centre, University of Saskatchewan. Other grain diets were formulated using barley, corn, and wheat as the only source of carbohydrate and soybean meal was included as the primary protein supplement. Acid insoluble ash (AIA) (Celite Corporation, Quincy, WA, USA) was used as an indigestible marker to allow for the determination of energy retention and apparent protein digestibility. The nutrient profiles of ingredients were based on Wang and Daun (2004), NRC (1994) and Degussa (2006).

6.3.2.1. Feed Processing

Pea and cereal grains were ground in a full circle pulverator–hammer mill (Model 160–D, Jacobson Machine Works, Minneapolis, Minn. 55427, USA) fitted with one of two different screen–hole sizes (3.2–, 6.4–mm). Afterward, feed ingredients for each experimental diet were mixed using a bakery mixer (Hobart mixer, Model L–800, Hobart Canada, Don Mills, ON. M3B 1B1) and then each mix was divided into two equal batches. One batch was pelleted (described later) and the other batch was fed in mash form. Pelleted diets were crumbled with a roller mill prior to feeding. Representative samples were collected from all diets for chemical analyses.

6.3.2.2. Pelleting Process

Pelleted diets were processed using a double pass conditioner pellet mill (CPM–Laboratory pellet mill, Model CL–5, California Pellet Mill Company, Crawfordsville, Indiana, USA) in the College of Engineering, University of Saskatchewan. Feed was held in a hopper until a vibratory feeder delivered it into the first conditioner in a controlled manner. A supply line delivered steam to the first of two conditioners (102.7–mm inside diameter and 830–mm length). The steam pressure and the speed of the mixing paddle in the conditioner were adjusted to establish desired conditions. The feed was held in the conditioner for approximately 60 s and then fed into the pelleter by a screw feeder. The pelleter consisted of a rotating ring die (4.5 mm holes and 45 mm thickness) and stationary roller, and the roller distributed and compressed the conditioned feed into the die holes. Conditioning temperatures were measured and recorded using thermocouples placed throughout both conditioners, the pelleter feeder, as well as at the outlet post–pelleting. The condition and pelleting processes was recorded in a computer using

Pelletmon software (Pelletmon, Steam pelleter monitor, Datlogger program, Version 2.07, September 1997). Pelleted feed was spread on trays and cooled and dried using forced air for 20 min at ambient temperature before being bagged. All diets were stored at room temperature until the experiment was conducted. The conditioning temperature for all diets averaged approximately 70°C and the production rate of pelleted feed was 30 kg h^{-1}.

6.4. Data Collection

6.4.1. Performance Data

Feed intake (**FI**) and bird weight were recorded on a cage basis at d 14 and 21. Body weight gain (**BWG**) and feed conversion ratio (**FCR**) were calculated for the same period. Mortality was recorded during the course of the experiment. Body weights of dead birds were used to correct FCR values.

6.4.2. Excreta Collection

Excreta were collected for 48 h at 20 and 21 d of age. Clean excreta trays covered with plastic sheets were placed under each battery cage and excreta was collected every 12 h (4 collections to minimize changes in excreta composition). For each excreta collection, feed and feather contaminants were removed and then excreta were placed in plastic bags and immediately frozen at –20°C. Subsequently samples were dried using a forced air oven (55ºC), pooled from the same replicate and treatment, and ground using a centrifugal laboratory mill (Retsch Mill ZM1, Newtown, PA, USA) fit with 1.0–mm screen–hole size. Collected excreta samples were used to determine AME and AME_n.

6.4.3. Digesta Collection

The experiment was terminated on d 22, birds were euthanized by cervical dislocation, and the intestinal tract was removed. The jejunal and ileal sections were separated at Meckel's diverticulum. The posterior ileum was defined as the section half way between Meckel's diverticulum and around 2 cm anterior to the ileal–cecal junction. The ileum was split into two parts of equal length defined as anterior and posterior. The digesta content from posterior ileum was gently squeezed out (using a roller vial) directly into 100 ml snap–cap vial. Digesta samples were pooled by replicate and treatment and during collection held on ice. Digesta samples were subsequently stored at –20^{o}C and later freeze dried. After freeze drying, the samples were ground with a mortar and pestle and mixed thoroughly before analysis. Collected digesta samples were used to determine apparent ileal protein digestibility.

6.4.4. Chemical Analyses

Samples from diets, excreta, and ileal digesta were analyzed for dry matter, AIA, gross energy, and nitrogen (protein = N × 6.25). Moisture was determined using standard procedures (AOAC, 2006) and AIA was determined using a modified procedure from Vogtmann et al. (1975). This procedure involved weighing approximately 1 to 2 g of samples into 16 × 125 mm disposable tubes and ashed at 500°C for at least 24 h (until the sample turns to white ash). After ashing, 5 mL of 4N HCl was added and thoroughly mixed, and then heated at 120°C for one hour. Then samples were centrifuged at 2500 g for 10 minutes and incubated at 80°C overnight. After drying, the samples were ashed again at 500°C overnight. Gross energy was determined according to AOAC (2006) using an oxygen bomb calorimeter (Model 1281; Parr Instruments, Moline, IL, USA)

standardized with benzoic acid. The nitrogen content was analyzed by a Leco–FP–528 protein analyzer (Model 601–500–100, Serial # 3211, Leco Corporation, St. Joseph, MA, USA). The chemical analyses of all samples were performed in duplicate except AIA, which was completed in quadruplicate.

6.4.5. Energy Retention Calculation

The gross energy (GE), nitrogen (N), and AIA content of diets and excreta were used to determine AME and AME_n using the following equations with appropriate corrections for differences in dry matter (DM) content.

$$AME_{n\ (cal/g.diet)} = AME_{cal/g.diet} - (8220 \times ANR_{g/g.diet})$$

$$AME_{cal/g.diet} = GE_{cal/g.diet} - [GE_{cal/g.excreta} \times (AIA\%\ diet \div AIA\%\ excreta)]$$

$$ANR_{g/g.diet} = N_{g/g.diet} - [N_{g/g.excreta} \times (AIA\%\ diet \div AIA\%\ excreta)]$$

Where GE is gross energy, N is nitrogen, AIA is acid insoluble ash, $ANR_{g/g.diet}$ is apparent nitrogen retained (g/g of diet), and 8220 is correction factor (cal) per g nitrogen retained in the body (Hill and Anderson, 1958).

6.4.6. Protein Digestibility Calculation

The crude protein and AIA data of experimental diets and ileal digesta were used to calculate the apparent ileal protein digestibility (AIPD) using the following equation:

$$Digestibility\ \% = 1 - [(AIA\%_{diet} \div AIA\%_{digesta}) \times (Nutrient\%_{digesta} \div Nutrient\%_{diet})] \times 100$$

6.5. Statistical Analysis

The experiment design was a randomized complete block design (RCBD) with three blocks (trials) and two replicates for each dietary treatment per block. In total, each dietary treatment was applied to 6 replicates (cages) with 6 birds per replicate. The experimental unit was the cage. Data from pea cultivars and cereal grains were analyzed

separately. Pea data were subjected to a three–way analysis of variance (2 screen–hole sizes; 3.2– or 6.4– mm × 2 feed form; mash or pellet × 9 pea cultivars). Cereal grain diets data were subjected to a three–way analysis of variance (2 screen–hole sizes; 3.2– or 6.4– mm × 2 feed form; mash or pellet × 3 cereal grain, barely, corn, and wheat). Each set of data was analyzed using the MIXED procedure of SAS 9.2 software (SAS, 2008) with blocks as a random factor. Data were checked for normality using PROC UNIVARIATE of SAS 9.2 prior to analysis. Treatment means were separated using Tukey's studentized range procedure test and differences were considered significant when $P \leq 0.05$ unless otherwise stated.

6.6. Results

Because block was not significant, it was removed from the model. The data from the three trials was combined and statistically analyzed.

6.6.1. Bird Performance

There were no interactions between the screen–hole sizes, feed form, and pea cultivar for all response criteria in chicken performance. Therefore, only the main effects are presented. The growth performance responses to pea cultivar, screen–hole size, and feed form of broiler chickens are presented in Table 6.2. Pea cultivar had no effect on the BW and FI but the BWG and FCR were affected. DS Admiral, Eclipse, and CDC Minuet had higher BWG than Alfetta, while values for other cultivars were intermediate and not different from all other cultivars. Similarly, Eclipse and CDC Minuet had lower FCR values than Alfetta, and all other cultivars resulted in intermediate values. Chicks given fine pea–based diets (3.2 mm screen–hole size) grew faster and more efficiently than those fed coarse diets (6.4 mm screen–hole size). FI was not affected by screen–hole size

of grind. Chicks given pelleted pea–based diets consumed more feed, had higher BWG, and lower FCR than those fed mash diets.

The growth performance responses to cereal grain, screen–hole size, and feed form of broiler chickens are presented in Table 6.3. FI was affected by grain with chicks fed the corn–based diet consuming the less feed than chicks fed the wheat–based diet; consumption of the barley–based diet was intermediate and not different than either of the other two grains. Wheat fed birds grew faster than chicks fed the corn– or barley–based diets. FCR was higher for the barley–based treatment in comparison with the other grains. Screen–hole size had no effect on FI, but BWG and FCR were improved feeding coarse diets (6.4 mm). Feed form did not affect FCR, but FI and BWG were increased feeding pelleted vs. mash diets.

6.6.2. Energy Retention

Energy retention was measured as traditional AME as well AME_n based on diet and excreta data. The AME and AME_n responses to pea cultivar, screen–hole size, and feed form of broiler chickens are presented in Table 6.4. AME and AME_n were affected by pea cultivar and there were 169 and 153 kcal/kg differences between the highest and the lowest value cultivar, respectively. Eclipse had the highest AME and AME_n on a dry matter basis with 2,770 and 2,570 kcal/kg, respectively. In contrast, Alfetta produced the lowest values with 2,600 and 2,416 kcal/kg, respectively. Overall, Eclipse produced higher AME and AME_n than DS Admiral, Alfetta, CDC Minuet, CDC Montero, Nitouche and SW Salute; CDC Mozart and CDC Striker had intermediate values. Pea AME and AME_n was increased by fine grind size (3.2– comparing with 6.4–mm screen–hole size).

On average, AME increased by 7.5% and AME_n by 7.9%. Pelleting improved AME by 20.5% and AME_n by 20.9% over all pea cultivars.

Significant interactions were found between pea cultivar and screen–hole size and pea cultivar and feed form. For example, the highest AME and AME_n values, 3,026 and 2,808 kcal/kg were reported with Eclipse, ground using 3.2 mm screen–hole size, and fed in pelleted form; respectively. Whereas the lowest values (2,040 and 1,872 kcal/kg for both techniques; respectively) were reported with DS Admiral, ground using 6.4 mm screen–hole size, and fed in mash form.

The AME and AME_n responses to cereal grain, screen–hole size, and feed form of broiler chickens is presented in Table 6.5. AME and AME_n values were affected by starch source. The barley–based diet had lower values than corn– and wheat–based diets. Coarse grinding (6.4 mm screen–hole size) improved AME and AME_n compared with fine grind size (3.2 mm screen–hole size). Interaction was found between starch source and screen–hole size for AME and AME_n values, however, only corn– and barley–based diets varied in AME and AME_n in regard to the screen–hole size, whereas values for wheat–based diet showed no significant differences. Feeding mash or pelleted form had no effect on AME and AME_n of studied cereal grains.

6.6.3. Protein Digestibility

Pea cultivar affected AIPD with Eclipse resulting in a higher digestibility than CDC Montero; all other cultivars are intermediate in value and are not significantly different from Eclipse and CDC Montero (Table 6.4). The hammer–mill screen–hole size of 3.2 mm increased AIPD in comparison with the 6.4 mm screen–hole size. Also, feeding pelleted diets increased AIPD in comparison to mash diets. The effect of pea

cultivar, screen–hole size, and feed form were independent and there was no interaction on protein digestibility.

AIPD values for barley–, corn–, and wheat–based diets are shown in Table 6.5. Cereal grain diets had reduced AIPD with the lower value for barley, 73.9%. Coarse grinding (6.4 mm screen–hole size) and feeding a mash diet increased AIPD compared with fine grinding (3.2 mm screen–hole size) and feeding pelleted diets, respectively.

6.7. Discussion

The present study was designed to examine the effects of feed processing and pea cultivar or cereal grain on energy value and apparent ileal protein digestibility in two experiments using broiler chickens. The performance data were presented to frame the conditions under which the experiment was conducted. It should be noted that the experimental pea diets were only supplemented with DL–Met. For that reason, other amino acids in pea–based diets may have become limiting and thereby affected bird performance. This finding is supported by BWG values that were lower and FCR values that were higher than the breed standard. However, FI, BWG, and FCR of broiler chickens show that the birds were eating feed and gaining weight during the experimental period and thereby unlikely to have impacted nutrient digestibility.

Pea cultivar had a significant effect on BWG and FCR as has been reported (Igbasan and Guenter, 1996). Moreover, feeding pelleted and fine ground pea–based diets increased BWG and improved FCR compared with those birds maintained on mash and fed coarse diets. These improvements can be explained by the beneficial effect of feed processing on nutrient digestibility.

In poultry diets, starch and protein are the major energy–yielding components. Starch supplies more than 50% of ME value which is positively correlated with starch digestion (Rogel et al., 1987; Carré et al., 1998; Wiseman et al., 2000). Therefore, factors that affect starch digestibility such as the size and structure of starch granules, amylose/amylopectin ratio, degree of crystallinity, and lipid and protein encapsulation can impact the contribution of starch to diet ME. Protein also contributes energy to poultry. As pea seeds contained an average of 230 g/kg crude protein, protein content and digestibility may have had an important impact on energy value for pea.

Grinding and pelleting are the most common used feed processing in poultry diets. These feed processing may affect starch digestibility by altering physic–chemical characteristics of starch. It also may have impact on other components in diet such as protein. The hydro–thermal effect of pelleting may cause some starch gelatinization (Svihus et al., 2004).

In poultry diets, the significant effect of pea cultivar on energy value was reported in earlier studies (Carré et al., 1991; Igbasan and Guenter, 1996). In this study, the value of AME and AME_n for pea obtained with 22–day–old broiler chicks was lower than those documented by Carré et al. (1991), but comparable to the result of Igbasan and Guenter (1996) and also in agreement with Igbasan et al., (1997) thought methodologies used were different. The differences in AME and AME_n values for pea cultivars may be related to differences in the chemical and physical structure of starch. Our results showed that the AME and AME_n values of pea were the lowest for coarse diets (6.4 mm) compared with fine grinding diets (3.2 mm). The differences between coarse and fine grind size could explain the differences in AME and AME_n values; therefore, nutrient

digestibility (mainly starch) would be the major factor affecting AME and AME_n variation. In agreement with earlier studies (Carré et al., 1991), this experiment demonstrated that pelleting has a positive impact on pea ME value. These results would confirm that nutrients digestibility is the main factor that affect ME value of pea for poultry. The positive effect of pelleting on pea AME values was reported to be mainly as a result of starch digestibility improvement (Moran et al., 1968; Carré et al., 1987, 1991).

The effect of screen–hole size on energy value in cereal grain–based diets was different than found for pea–based diets. The 6.4 mm hammer–mill screen–hole size increased energy value compared to 3.2 mm. This result is in agreement with Preston et al. (2000) and Svihus et al. (2004).

The results of the current research demonstrate that pea cultivars are more sensitive to feed processing than cereal grains (Figure 6.1). For example, fine grinding (3.2 mm screen–hole size) vs. coarse grinding (6.4 mm screen–hole size) resulted in 6.2% improvement in AME_n of pea–based diets, whereas only 2.4% difference was found for grain–based diets. Moreover, the effect of pelleting was more pronounced in pea–based diets than grain diets. AME_n of pelleted pea–based diets was higher than mash form by 16.3%, in contrast pelleted grain–based diets had no effect on AME_n.

The protein digestibility of pea in poultry has not been extensively researched. The average of AIPD in pea diets varied between 65.9 and 82.2%. The AIPD obtained in this research was lower than those published by Brenes et al. (1993) and Igbasan and Guenter (1996). The cause of these differences might be as a result of methodology applied in determining protein digestibility. The effect of pea cultivar on AIPD was in agreement with Carré et al. (1991) and Igbasan and Guenter (1996). Small screen–hole

size and pelleting induced a positive effect on AIPD values of pea–based diets for poultry. These results confirm the reported by Carré et al. (1991) and Crevieu et al. (1997). It can be speculated that the 1.5 mm screen–hole size of hammer mill had neutralized the effect of pelleting.

Protein digestibility of grains should be discussed with caution as soybean meal was included in cereal–grain based diets. The effect of feed processing on protein digestibility of grains was the opposite to that found for pea–based diets.

It is concluded from this experiment that significant differences exist in AME, AME_n, and AIPD values between pea cultivars. These findings also show that these parameters can be improved by grinding pea seeds using a hammer–mill with a 3.2 mm screen–hole and pelleted at 70°C. It was shown also that the responses of pea–based diets to feed processing were more pronounced than cereal grain–based diets. Based on these results, further studies are warranted to evaluate the impact of pea cultivar, hammer–mill screen–hole size and pelleting on starch and amino acids digestibility of pea.

TABLE 6.1. Ingredient composition and calculated nutrient content (g/kg) of the starter diet and experimental diets fed from 14 to 22 d of age

Ingredient	Starter diet (0 to 13 d)	Experimental diets			
		Pea	Barley	Corn	Wheat
Pea	100.0	871.1	–	–	–
Barley	50.0	–	576.8	–	–
Corn	266.9	–	–	542.2	–
Wheat	100.0	–	–	–	590.0
Soybean meal (48%)	388.1	–	316.3	375.8	316.5
Canola oil	50.0	70.0	52.1	26.8	39.0
Di–calcium phosphate[1]	19.3	17.2	15.8	17.4	16.3
Ground limestone	12.3	11.6	11.8	11.1	11.6
Celite–insoluble ash[2]	–	15.0	15.0	15.0	15.0
Vitamin–mineral premix[3]	5.0	5.0	5.0	5.0	5.0
Sodium chloride	4.7	4.5	4.7	4.9	4.4
Choline chloride (60%)	1.0	1.0	1.0	1.0	1.0
DL–Methionine	2.6	4.6	1.5	0.8	1.2
Avizyme 1302[4]	0.1	0.1	0.1	0.1	0.1
Calculated nutrient content					
AME[5] (kcal/kg)	3,030	2,925	2,900	3,000	3,000
Crude protein (N × 6.25)	249.8	191.6	221.3	229.6	245.1
Calcium	10.0	9.0	9.0	9.0	9.0
Available phosphorus	5.0	4.5	4.5	4.5	4.5
Starch	340.0	418.1	314.4	401.3	365.9
Linoleic acid	14.7	19.7	12.8	19.4	13.4
Sodium	2.1	2.1	2.1	2.1	2.1
Potassium	9.9	8.6	9.3	8.8	8.4
Chloride	3.1	3.3	4.4	4.0	3.4
Digestible Methionine	5.5	5.8	4.4	3.9	4.3
Digestible Met + Cys	8.7	7.5	7.6	7.0	7.9
Digestible Lysine	12.5	11.2	10.5	11.1	10.5
Digestible Tryptophan	2.7	1.1	2.4	2.5	2.8
Digestible Threonine	7.8	5.4	7.1	7.5	7.3
Digestible Isoleucine	9.2	5.9	8.2	8.7	8.9
Digestible Valine	9.8	6.3	9.2	9.5	9.8
Digestible Arginine	15.8	14.0	13.1	14.3	14.2

[1] Di–calcium Phosphate: 15 % Ca; 21% P.
[2] Celite Corporation, Quincy, WA, USA.
[3] Supplied per kilogram of diet: vitamin A (retinyl acetate + retinyl palmitate), 11000 IU; vitamin D, 2200 IU; vitamin E (dl–alpha–topheryl acetate), 300 IU; menadione, 2.0 mg; thiamine, 1.5 mg; riboflavin, 6.0 mg; niacin, 60 mg; pyridoxine, 4.0 mg; vitamin B_{12}, 0.02 mg; pantothenic acid, 10.0 mg; folic acid, 0.6 mg; biotin, 0.15 mg; Iron, 80 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.8 mg; selenium, 0.3 mg; and $CaCO_3$, 500 mg.
[4] Danisco Animal Nutrition, Marlborough, Wiltshire.
[5] National Research Council 1994.

TABLE 6.2. Growth performance of broiler chickens (14 to 22 d) fed pea based diets as affected by pea cultivar, screen–hole size, and feed form

	Body weight (g)	Feed intake (g)	Body weight gain (g)	FCR[1]
Pea cultivar[2]				
DS Admiral	1008.3	794.6	487.2[a]	1.63[ab]
Alfeta	975.3	799.6	445.1[b]	1.74[a]
Eclipse	1002.6	784.4	491.6[a]	1.61[b]
CDC Minuet	994.2	782.2	487.2[a]	1.62[b]
CDC Montero	985.5	794.0	477.7[ab]	1.66[ab]
CDC Mozart	988.3	777.2	467.1[ab]	1.63[ab]
Nitouche	998.3	793.2	476.9[ab]	1.65[ab]
SW Salute	1000.9	797.2	476.7[ab]	1.65[ab]
CDC Striker	998.9	784.5	476.4[ab]	1.64[ab]
Screen–hole size[3] (mm)				
3.2	1008.7[a]	795.2	488.8[a]	1.61[b]
6.4	980.7[b]	784.1	463.7[b]	1.69[a]
Feed form[3]				
Pellet	1038.7[a]	824.0[a]	519.9[a]	1.56[b]
Mash	950.7[b]	755.3[b]	432.5[b]	1.73[a]
Pooled SEM[4]	4.70	4.41	4.49	0.011

[a–b] Mean values within a column and main effect (pea cultivar, screen–hole size, and feed form) with different superscript letters are significantly different ($P < 0.05$).
[1] FCR–feed conversion ratio corrected for mortality.
[2] Each value represents the mean of 24 replicates with 6 birds each.
[3] Each value represents the mean of 108 replicates with 6 birds each.
[4] SEM–Standard error of the mean (n = 216).

TABLE 6.3. Growth performance of broiler chickens (14 to 22 d) fed barley–, corn–, wheat–based diets as affected by screen–hole size and feed form

	Body weight (g)	Feed intake (g)	Body weight gain (g)	FCR[1]
Cereal grain[2]				
Barley	984.5[b]	793.0[ab]	470.6[b]	1.69[a]
Corn	998.7[b]	759.5[b]	479.3[b]	1.51[b]
Wheat	1047.0[a]	809.6[a]	528.9[a]	1.49[b]
Screen–hole size[3] (mm)				
3.2	997.1	789.6	475.7[b]	1.60[a]
6.4	1023.0	785.2	510.2[a]	1.53[b]
Feed form[3]				
Pellet	1031.7[a]	814.1[a]	517.9[a]	1.56
Mash	988.4[b]	760.6[b]	467.9[b]	1.57
Pooled SEM[4]	8.03	7.75	7.30	0.017

[a–b] Mean values within a column and main effect (pea cultivar, screen–hole size, and feed form) with different superscript letters are significantly different ($P < 0.05$).
[1] FCR–feed conversion ratio corrected for mortality.
[2] Each value represents the mean of 24 replicates with 6 birds each.
[3] Each value represents the mean of 36 replicates with 6 birds each.
[4] SEM–Standard error of the mean (n = 72).

TABLE 6.4. AME, AME_n, and apparent ileal protein digestibility (AIPD) of pea fed to broiler chickens (14 to 22 d) as affected by pea cultivar, screen–hole size, and feed form

	AME (kcal/kg)[1]	AME_n (kcal/kg)[1]	AIPD (%)
Pea cultivar[2]			
DS Admiral	2612[b]	2423[c]	76.9[ab]
Alfeta	2600[b]	2417[c]	75.7[ab]
Eclipse	2770[a]	2570[a]	78.4[a]
CDC Minuet	2644[b]	2491[abc]	77.6[ab]
CDC Montero	2651[b]	2474[bc]	74.4[b]
CDC Mozart	2678[ab]	2487[abc]	77.7[ab]
Nitouche	2606[b]	2435[c]	76.8[ab]
SW Salute	2620[b]	2453[bc]	76.3[ab]
CDC Striker	2702[ab]	2520[ab]	76.7[ab]
Screen–hole size[3] (mm)			
3.2	2750[a]	2568[a]	78.8[a]
6.4	2557[b]	2381[b]	74.6[b]
Feed form[3]			
Pellet	2900[a]	2709[a]	80.7[a]
Mash	2407[b]	2240[b]	72.7[b]
Interaction (*P*–value)			
Cultivar*Screen–hole size	0.005	0.002	NS
Cultivar*Feed form	0.045	0.032	NS
Pooled SEM[4]	20.5	19.5	0.41

[a–c] Mean values within a column and main effect (pea cultivar, screen–hole size, and feed form) with different superscript letters are significantly different ($P < 0.05$).
[1] Based on dry matter.
[2] Each value represents the mean of 24 replicates with 6 birds each.
[3] Each value represents the mean of 108 replicates with 6 birds each.
[4] SEM–Standard error of the mean (n = 216).

TABLE 6.5. AME, AME_n (kcal/kg)[1], and AIPD (%) of barley–, corn–, and wheat–based diets fed to broiler chickens (14 to 22 d) as affected by screen–hole size and feed form

	AME (kcal/kg)[1]	AME_n (kcal/kg)[1]	AIPD (%)
Cereal grain[2]			
Barley	3196.9[b]	3037.9[b]	73.9[b]
Corn	3407.3[a]	3255.6[a]	80.9[a]
Wheat	3381.0[a]	3226.2[a]	80.3[a]
Screen–hole size[3] (mm)			
3.2	3287.3[b]	3135.6[b]	76.9[b]
6.4	3369.6[a]	3210.8[a]	79.9[a]
Feed form[3]			
Pellet	3316.8	3162.7	76.5[b]
Mash	3340.0	3183.7	80.3[a]
Interaction (*P*–value)			
Grain*Screen–hole size	0.0087	0.0195	NS
Pooled SEM[4]	18.40	17.77	0.57

[a–b] Mean values within a column and main effect (pea cultivar, screen–hole size, and feed form) with different superscript letters are significantly different ($P < 0.05$).
[1] Based on dry matter.
[2] Each value represents the mean of 24 replicates with 6 birds each.
[3] Each value represents the mean of 36 replicates with 6 birds each.
[4] SEM–Standard error of the mean (n = 72).

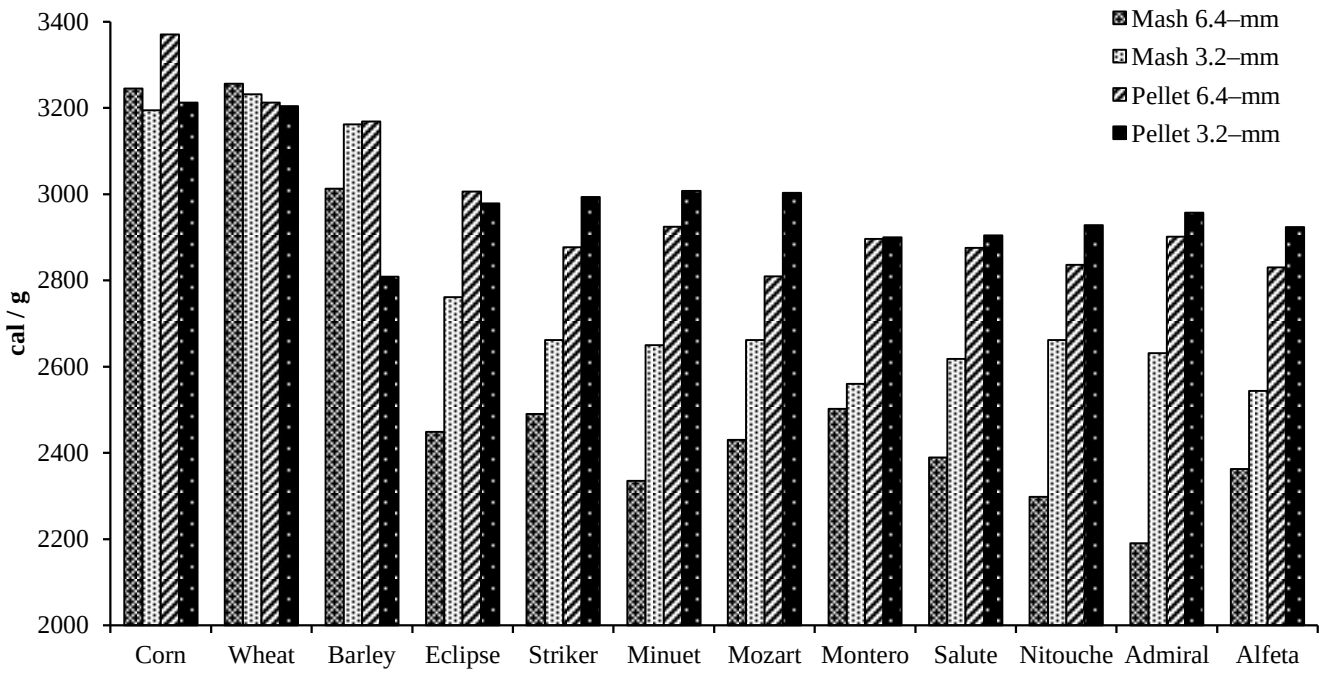

FIGURE 6.1. AMEn of cereal grain and pea cultivar based–diets as affcted by screen hole size and pelleting.

EFFECTS OF FEEDING SLOW DIGESTED STARCH FROM PEA ON CHICKEN PERFORMANCE AND METABOLISM

7.0. THE EFFECT OF DIETARY LEVEL OF FIELD PEA AND BALANCED AMINO ACIDS ON PERFORMANCE OF TWO STRAINS OF LAYING HENS

7.1. Abstract

Energy and amino acids are key nutrients in relation to laying hen performance. Starch is the major source of energy in poultry feed and it has been suggested that feeding a mixture of rapidly and slowly digested starch can improve amino acid utilization in poultry. In order to examine the effect of feeding slowly digested starch on the amino acid requirement of laying hens, a factorial experiment was conducted with two strains (Hy–Line CV 20 and Lohmann LSL–Lite), three levels of pea inclusion (0, 150, and 300 g/kg of diet), and three levels of lysine intake (700, 780, and 860 mg per day). Diets were formulated on a total amino acid basis and with ratios of dietary indispensable amino acids to lysine closely maintained. Strain affected most performance characteristics, but did not interact with dietary treatments. Neither pea nor lysine level affected feed consumption, egg production, eggshell quality, mortality, and numbers of soft–shelled, cracked, broken, double, and abnormal eggs. Body weight gain and egg weight increased with increasing levels of pea and lysine inclusion, but the interaction between these main effects was only significant for body weight gain ($P = 0.047$). Feed efficiency, as defined by feed intake per kg egg mass, improved as lysine intake increased ($P = 0.035$), but dietary pea level did not affect this trait. A progressive improvement in feather score was observed as the level of amino acid intake in the diet increased, whereas yolk color was improved as level of pea inclusion increased. In conclusion, pea inclusion up to 300 g/kg

in laying hen diets was well tolerated by laying hens and improved energy retention as indicated by increased body weight and egg weight. The results of this experiment did not confirm the hypothesis that slow digested starch from pea improves amino acid utilization in laying hens.

Key words: pea, amino acids, slowly degraded starch, laying hens

7.2. Introduction

Field pea (*Pisum sativum* L.) production in Western Canada is increasing, as farmers understand its agronomic advantages (Ratnayake et al., 2002). Although produced mainly for human food, surplus and poor quality pea are used in animal feed. The use of pea in poultry diets is based on its availability and competitive price. However, a clear understanding of the nutritional value of pea in poultry diets is required to maximize its inclusion and potentially reduce the cost of egg production.

A moderate level of AME and crude protein make pea a suitable feed ingredient for laying hen diets (Gatel, 1994; Castell et al., 1996). Pea seeds have high starch content and starch is the main dietary energy supply in poultry feed. Pea starch differs from cereal grains in terms of the rate and extent of starch digestion in the small intestine (Weurding et al., 2001, 2003a). Pea starch is less accessible to digestive enzymes than in cereal grains (Longstaff and McNab, 1987), and is therefore more slowly and poorly digested. In human nutrition, the slow rate of starch digestion (lower glycemic index) has been suggested to provide nutritional benefits (Jenkins et al., 2002; Björck, 2006), but the value of slowly vs. rapidly digested starches requires confirmation in animals.

Although pea inclusion in poultry feed has occurred for years, published studies regarding the acceptable level of pea inclusion in laying hen diets are contradictory. Moran et al. (1968) compared feeding a corn–soybean based diet to diets containing 150 or 300g/kg of pea to laying hens (28 to 40 wk of age). Feeding either level of pea reduced feed utilization, but other laying hen performance criteria were unaffected. However, steam–conditioning pea (90°C) improved feed utilization and maintained laying hen performance. Inclusion of 375 g/kg of heat processed pea (165°C for 50s) in oat–based diet maintained equal egg production in comparison to a fishmeal control diet, but egg size was reduced (Davidson, 1980). Castanon and Perez–Lanzac (1990) evaluated three levels of pea inclusion (166, 333, and 500 g/kg) in isoenergetic (2600 kcal/kg) and isonitrogenous (169 g/kg) diets. The highest level of pea inclusion reduced egg production by 8%. They concluded that the upper level of pea incorporation into laying hen diets without a reduction in productivity was 300 g/kg. In contrast, Ivusic et al. (1994) found that the inclusion of 590 g/kg of pea resulted in thinner egg shells and less yolk pigmentation than a corn–soybean meal diet, whereas egg production, feed conversion, and egg weight were not affected. They concluded that pea inclusion levels up to 445 g/kg supplemented with DL–methionine had no adverse effect on laying hen performance (22 to 58 wk of age). Igbasan and Guenter (1997a) reported that 400 g/kg of pea substitution in a corn–soybean meal diet did not affect the performance of laying hens. In another study, Igbasan and Guenter (1997b) found that 600 g/kg of dietary inclusion of pea did not affect feed intake, egg weight and albumin quality, but reduced egg production, FCR, and egg mass. However, such adverse effects were eliminated when pea was micronized. Perez–Maldonado et al. (1999) reported that up to 250 g/kg of

pea inclusion in a laying hen diet maintained excellent performance (26 to 66 wk of age). More recently, Fru–Nji et al. (2007) replaced soybean and wheat in laying hen diets with six levels of pea inclusion (0, 100, 200, 300, 400, and 500 g/kg). The study started at 24 wk of age and lasted for 52 weeks. They found that pea inclusion up to 500 g/kg had no adverse effect on egg production, egg weight, and FCR, but feed intake increased and body weight gain decreased as the level of pea inclusion increased. They suggested that anti–nutritional factors may have interfered with nutrient digestibility and therefore reduced energy and amino acid availability. As a result of the differences in published results, poultry producers and feed manufacturers may unnecessarily limit pea use in laying hen diets.

In human nutrition, starch has been characterized as rapid digested (**RDS**), slow digested (**SDS**), and resistant to digestion (**RS**) (Englyst et al., 1992). SDS sustains and stabilizes blood glucose levels after a meal and thereby moderates the glycemic index (GI), which is linked with health benefits such as reduced risk of cardiovascular disease and diabetes (Jenkins et al., 2002). In turn, level of blood insulin is well correlated with blood glucose (Björck, 2006) and can influence a number of metabolic actions including protein synthesis (Proud, 2006). In poultry, dietary energy is mainly supplied by starch and in most cereal grains, starch is digested rapidly in the upper part of the small intestine, whereas other nutrients such as protein are not digested or absorbed until they reach the ileum. RDS causes a spike in blood glucose level, which could results in insulin induced consequences on energy and amino acid metabolism, and protein utilization and deposition in body tissue. In addition, when glucose is available as an energy substrate for enterocytes in the proximal small intestine, amino acids are the source of energy for

the distal small intestine. As a consequence, more amino acids are catabolized thereby making less amino acid available for protein synthesis. The slowly digested nature of pea starch has been suggested to have a unique nutritional value for poultry (Weurding et al., 2003a,b) with evidence that the presence of slow degraded starch reduces the amino acid requirements of broilers and that a mixture of rapidly and slowly degraded starch improves broiler productivity in contrast to diets containing only rapid digested starch.

Although considerable research has been completed on the inclusion of pea in laying hen diets, the lack of consistent results and short trial lengths support the need for further studies in this area. In addition, the authors were unaware of published research to determine whether slow digested starch from pea improves amino acid utilization by laying hens. Therefore, an experiment was conducted to investigate the relationship between levels of dietary pea and balanced amino acids in laying hen diets.

7.3. Materials and Methods

Experimental procedures were performed in accordance with the Guide to the Care and Use of Experimental Animals, Canadian Council on Animal Care (1993). The research protocol was reviewed by the Animal Care Committee of the University of Saskatchewan.

7.3.1. Birds and Housing

A total of 1350 pullets of two laying hen strains (675 each of Hy–Line CV–20 and Lohmann LSL–Lite) were housed in a cage lay facility of commercial design at the Poultry Centre, University of Saskatchewan. During the experiment, a minimum ambient temperature of approximately 21°C was maintained. Three pullets were placed in each cage (cage dimensions 30.5 × 46 cm with a height of 52 cm, floor space = 468 cm^2/hen).

Day length was increased at 18 wk of age from 8L:16D to 14L:10D where it remained throughout the experiment with a light intensity of 5 lx. During the pre–experimental period (i.e., up to 20 wk of age) pullets were fed ad libitum a commercial laying hen diet in mash form. From 20 to 56 wk of age, hens from each strain were randomly assigned to be fed 1 of 9 experimental diets. Free access to feed and water was provided during the trial.

7.3.2. Experimental Diets

A phase–feeding program was used in this study to maintain levels of lysine intake as hens increased feed intake during the laying period. The experimental diets in each phase were formulated based on hen nutrient requirement and actual feed intake, and there were four phases used in this experiment (Table 7.1). In each phase, nine isoenergetic (2,850 kcal/kg) diets were formulated to meet or exceed the daily nutrients requirement for laying hens (NRC, 1994), but with modified levels of amino acids. There were three levels of pea inclusion (0, 150, and 300 g/kg of diet) and three levels of lysine intake 90, 100, and 110% of NRC recommendation (700, 780, and 860 mg/h/d; respectively). Pea replaced wheat and soybean meal and diets were formulated on a total amino acid basis with dietary essential amino acids maintained at a constant ratio to lysine content (ideal protein). The ratios of indispensable amino acids to lysine are presented in Table 7.2.

The ingredient composition and nutrient content of the phase experimental diets are shown in Tables 7.3 through 7.6. The calcium supply was equal in all dietary treatments (38 g/kg) and available phosphorus was adjusted based on feed intake. Feedstuffs were ground using a Jacobson hammer–mill (Model 170F8, Jacobson

Machine Works, Minneapolis, Minn. 55427, USA) with 6.35 mm screen–hole size before mixing, and diets were fed in mash form. The cultivar of pea used in this study was Eclipse and wheat was feed grade of unknown cultivar. The same samples of all feedstuffs were fed throughout the trial.

7.3.3. Data Collection

Body weight of all birds was recorded individually at 20, 44, and 55 wk of age. Feed consumption was determined on a replicate basis (15 hens) at the end of each 28–d period. Egg production was recorded on a replicate basis from Monday to Friday for the complete length of the experiment. Eggs were scored for cleanliness, and classified as normal, double–yolk, soft–shelled, cracked, broken, and abnormal (small or misshapen eggs). Prior to statistical analyses, the 5–d egg numbers per wk were converted mathematically to 7–d per wk basis (5–d egg number × 1.4). Total hen housed egg production (**THHP**) and total hen day egg production (**THDP**) were calculated as a percent of hen housed and hen day numbers, respectively. Egg weight and specific gravity were assessed on a replicate basis on all collected eggs produced on the last day of each 28–d period. Egg weight was measured on the day of collection whereas specific gravity was determined on the morning of next day. Eggs were stored at room temperature overnight. Specific gravity of eggs was evaluated using the flotation method with 9 saline solutions ranging from 1.060 to 1.100 g/cm^3 in 0.005 increments. NaCl solutions were calibrated before each test. Feed efficiency was calculated on a total feed to egg mass (**TFEM**) and total feed to dozen of egg (**TFDE**) basis. TFEM was calculated based on data from each 28–d period, egg number, egg weight, and feed consumption, and described as g of feed consumed per g of egg produced. TFDE was calculated for the

same periods based on feed intake in kg and egg numbers. Yolk color was determined using the Roche yolk color fan on a one day egg collection at 55 wk of age. Individual bird feather condition was subjectively scored at 55 wk of age on 5 areas (neck, wings, back, vent, and breast) using the procedure of Davami et al. (1987), where scores range from 1 (very poor feathering) to 4 (full plumage). Dead birds were collected and weighed daily, and mortality was recorded on a replicate basis.

7.4. Statistical Analysis

The experiment design was a completely randomized design (CRD). The experimental data were analyzed as a 2 × 3 × 3 factorial arrangement with the main effects of two laying hen strains, three levels of pea inclusion (source of SDS), and three levels of amino acid (Lys) intake. Each combination of strain, pea inclusion, and amino acid intake was replicated 5 times with 5 adjoining cages per replicate and 3 hens per cage. The experimental unit included 15 hens. Data were checked for normality using PROC UNIVARIATE and transformed as needed. Data were subjected to analysis of variance using the MIXED procedure of SAS 9.2 software (SAS Institute, 2008). When ANOVA indicated a significant treatments effect, Tukey's Studentized Range Test was used for mean separation and pdmix800 was used to provide letters for differences (Saxton, 1998). The level of significance was fixed at $P \leq 0.05$ unless otherwise stated. Data were also tested for regression relationships with pea inclusion using PROC Reg (Regression) and PROC RSReg (Response Surface Regression) of SAS 9.2 software.

7.5. Results

The same strain and dietary effects were found during all four phases of feeding, therefore only overall data are presented. Strain had a significant effect on all the studied performance characteristics but the two strains of hens responded similarly to levels of pea inclusion and amino acid intake (Table 7.7, 7.8). Lohmann LSL–Lite hens had higher feed intake, THHP, THDP, egg weight, egg specific gravity, TFEM, TFDE, and mortality; whereas Hy–Line CV–20 hens had higher body weight gain, darker egg color, and feather score. Over all the studied parameters, there were no interactions between dietary treatments except for body weight gain ($P < 0.047$).

Lohmann LSL–Lite hens were heavier (1.3 and 3.7% at 20– and 44–wk, respectively) than Hy–Line CV–20 hens, but by the end of the trial (54 wk) both strains had a similar body weight (Table 7.10). Therefore, Hy–Line CV–20 hens had higher body weight gain. Over the entire production trial, Lohmann LSL–Lite hens consumed more feed (~10g/h/d) (Table 7.9) and produced ~4% more eggs on a per hen–housed and per hen–day basis than Hy–Line CV–20 hens (Table 7.11, 7.12). Lohmann LSL–Lite had a higher egg weight up to 38 wk of age, while Hy–Line CV–20 hens produced a larger egg than Lohmann LSL–Lite strain (Table 13). Egg specific gravity was greater with Lohmann LSL–Lite during the overall production cycle. Strain had a significant effect on TFEM and TFDE with Hy–Line CV–20 having a lower ratio (Table 15, 16). Hy–Line CV–20 birds had a higher feather score and yolk color compared with Lohmann LSL–Lite hens. Lohmann LSL–Lite strain had higher mortality (3.18%) in contrast to Hy–Line CV–20 (0.59%). Cracked egg numbers were higher for Hy–Line CV–20 birds and double

yolked eggs higher for the Lohmann LSL–Lite strain (Table 7.8). Other egg characteristics such as soft, broken, and abnormal egg were not affected by strain.

The effects of dietary treatments on performance characteristics of laying hens are presented in Tables 7 and 8. Neither pea inclusion nor daily lysine intake affected hen feed intake, THHP, THDP, and specific gravity. However, body weight gain, and egg weight increased in a linear fashion with increasing levels of pea inclusion or amino acids intake. Feed efficiency was measured as total feed to egg mass ratio (TFEM) and total feed per dozen of eggs (TFDE). No significant dietary effects on TFDE were observed for the overall production cycle. TFEM was improved by increasing lysine level, but pea inclusion had no effect (Table 7.7). Feather score at trial end improved (linear regression) with increasing lysine intake, but level of pea inclusion had no effect (Table 7.8). Egg yolk color darkened with increasing level of pea, but was unaffected by lysine intake. Egg yolk color increased from 2.3/9 with no pea in diet to 4.2/9 with 300g/kg dietary pea (Table 7.8). The numbers of soft–shelled, cracked, broken, double, and abnormal eggs were not affected by pea inclusion or lysine levels. Finally, none of the dietary treatments significantly affected mortality, which averaged 1.87% overall production cycle (20 – 56 wk). Feeding 150 and 300 g/kg of pea to laying hens combined with three levels of amino acids intake (90, 100, and 110% of NRC recommendation) did not depress hen performance compared to birds fed wheat–based diet. Strain of hen responded similarly to dietary treatments and the effects of pea inclusion (source of SDS) on productive performance of laying hens was independent from the level of amino acids intake except for body weight gain.

7.6. Discussion

Laying hens should be fed diets, which provide a consistent daily intake nutrient during the laying period. As egg production and body weight increase during a laying cycle, hens tend to consume more feed. Therefore using the same diet for the whole production period will result in too little or too much daily nutrient intake. The laying hen diets in this study were formulated based on phase feeding approach with four phases based on hen feed intake, and therefore a more accurate daily nutrient intake was achieved.

In the small intestine, starch is gradually degraded and pea starch is more slowly digested compared to wheat starch (Weurding et al., 2001; Ebsim et al., 2013). The main objective of this study was to investigate whether the effect of amino acid intake on laying hen performance depended on the level of slow digested starch from pea. A positive effect of slowly digested starch on hen performance was hypothesized based on human nutrition research (Jenkins et al., 2002; Björck, 2006) and broiler experiments (Weurding et al., 2003a, b). Our hypothesis was that including SDS from pea in laying hen diet would improve amino acid utilization and would reduce the amino acid requirement. However, the lack of interaction between dietary treatments, pea inclusion (source of SDS) and level of amino acid intake was not supportive of our hypothesis.

Because laying hen requirements for energy and protein are lower than broilers, more opportunity exists to formulate laying hen diets with pea than for other types of poultry. Feed intake, egg production, feed conversion, egg shell quality (specific gravity), numbers of soft–shelled, cracked, broken, double, and abnormal eggs, feather score, and

mortality in pea based diets were similar to the wheat based diets. The present experiment confirms that inclusion up to 300 g/kg of pea in laying hen diets supplemented with varied levels of lysine did not have any adverse effects on egg production or egg quality. These results are in agreement with previous studies (Moran et al., 1968; Castanon and Perez–Lanzac, 1990; Ivusic et al., 1994; Igbasan and Guenter, 1997a; Perez–Maldonado et al., 1999; Fru–Nji et al., 2007). Moreover, similar to the findings of Igbasan and Guenter (1997a,b), this study showed that egg yolk color improved with the level of pea inclusion and eggshell quality was not reduced. The improvement in egg yolk color may be related to the quantity of xanthophyll in pea; however, information supporting these results is scarce.

Laying hens fed diets containing 150 or 300 g/kg of pea had higher body weight at trial end, body weight gain (20 – 56 wk), and higher egg weight output than those fed wheat based diets. This result may be as a consequence of improved energy utilization. It can be hypothesized that SDS from pea provides more glucose to the distal part of the small intestine, which may have spared the use of amino acids as a source of energy (Björck, 2006). Moreover, a better–balanced nutrient profile in pea diets may have advanced impact on laying hen performance. For instance, linoleic acid, arginine, and branched amino acids were slightly higher in pea–based diets than wheat based diets.

The reasons for the lack of interaction between the level of slowly digested starch and amino acids intake are not completely clear. It may be the ratio of pea starch to cereal starch was not wide enough. The lowest level of amino acid intake tested was based on 90% of the NRC recommendation. In this research, the 90% level of amino acids had no negative effect on most performance data. This level may not have been low enough to

show the effect of SDS on amino acids availability. Moreover, in this study diets were formulated based on the total amino acids as well the ideal protein concept. Therefore, in addition to methionine amino acids other than were supplemented in the experimental diets. The amino acid data that were used in diet formulations may have been underestimated and pea may have provided more amino acids than anticipated. Because laying hens have lower amino acid requirement than young broilers, better digestibility as adults, and are less sensitive to the effect of ANFs than broilers, the effect of SDS on amino acid availability might be less pronounced.

The present study showed no effect of lysine intake (90, 100, and 110% of NRC recommendation) on feed intake, THHP, THDP, egg quality, and TFDE, whereas body weight at trial end, body weight gain, egg weight, TFEM, and feather score were improved as lysine intake increased. It can be speculated that the NRC recommendation of amino acid requirement for laying hens is not adequate to support maximum egg weight and superior feed efficiency.

In this experiment, the regression approach was used to estimate the effect of pea inclusion and amino acid intake on laying hen performance (Table 9). It was not surprising that significant equations were found between amino acid intake and body weight gain and egg weight. The significant linear regression between pea inclusion and body weight and egg weight confirms the suitability of pea as a feedstuff for laying hens (Gatel, 1994; Castell et al., 1996). However, the effect of pea inclusion on egg weight cannot be fully explained.

In conclusion, the long–term feeding of up to 300 g/kg of pea (SDS source) with varied levels of amino acid intake (90, 100, and 110% of NRC) had no detrimental

effects on the performance of two strains of laying hen. The strains differed in performance, but responded similarly to pea inclusion and amino acid intake. Increases in body weight gain and egg weight with increasing pea level indicate a beneficial effect of feeding pea that is not accounted for by nutritional values used in feed formulation. The effect of SDS on amino acid availability cannot be confirmed, possibly due to the range in amino acid content and ratio of SDS:RDS not being wide enough. Therefore, further research with wide levels of amino acid intake higher levels of dietary pea inclusion is needed. The present study confirmed that pea could be included in laying hen diets as a source of supplemental energy and protein.

TABLE 7.1. Calculated level of lysine (mg/h/d) intake based on phase–feeding program

Phase–feeding		Feed intake (g/h/d)	As % of NRC recommendations		
#	Age (wk)		90%	100%	110%
I	20–23	90	778	867	965
II	24–31	100	700	780	860
III	32–42	105	667	743	819
IV	43–56	110	636	709	782

TABLE 7.2. Target ratios of indispensable amino acids to total lysine (g/g) in all experimental diets

Amino acids	Ratio
Methionine : Lysine	0.46
Met + Cys : Lysine	0.84
Threonine : Lysine	0.76
Tryptophan : Lysine	0.23
Arginine : Lysine	1.17
Leucine : Lysine	1.34
Isoleucine : Lysine	0.74
Valine : Lysine	0.88

TABLE 7.3. Ingredient composition and calculated nutrient of experimental diets for feeding phase I (20 to 23 wk of age) based on 90 g/h/d feed intake

Ingredient (g/kg)	90% of NRC			NRC			110% of NRC		
Wheat	728.7	565.9	426.3	678.2	515.5	385.0	627.8	465.1	343.8
Pea	0.0	150.0	300.0	0.0	150.0	300.0	0.0	150.0	300.0
Soybean meal	112.3	114.8	95.5	157.5	160.0	132.0	202.7	205.3	168.5
Canola oil	28.2	40.5	50.3	33.4	45.8	54.4	38.6	51.0	58.6
Limestone	103.5	103.2	103.1	103.2	103.0	102.9	103.0	102.7	102.7
Dicalcium phosphate	12.7	12.8	12.9	12.7	12.8	12.9	12.7	12.8	12.9
Sodium chloride	2.7	2.9	3.1	2.8	3.0	3.1	2.9	3.1	3.2
L–Lysine HCL	2.7	1.0	0.0	2.4	0.7	0.0	2.2	0.5	0.0
DL–Methionine	1.5	1.5	1.7	1.9	1.9	2.2	2.3	2.3	2.6
L–Threonine	1.2	0.8	0.6	1.3	0.8	0.8	1.3	0.8	0.9
L–Tryptophan	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.2
Choline chloride	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin–mineral premix[1]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Enzyme[2]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Calculated nutrient (g/kg)									
AME (kcal/kg)	2,850	2,850	2,850	2,850	2,850	2,850	2,850	2,850	2,850
Crude protein (N × 6.25)	162.0	164.7	161.4	176.1	178.8	173.2	190.3	193.0	185.0
Calcium	42.2	42.2	42.2	42.2	42.2	42.2	42.2	42.2	42.2
Nonphytate phosphorous	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4
Linoleic acid	13.8	15.7	17.3	14.4	16.3	17.8	15.0	16.9	18.3
Lysine	7.8	7.8	7.8	8.7	8.7	8.7	9.6	9.6	9.6
Methionine	3.6	3.6	3.7	4.2	4.2	4.3	4.8	4.8	4.9
Met+Cys	6.5	6.5	6.5	7.3	7.3	7.3	8.0	8.0	8.0
Threonine	5.9	5.9	5.9	6.6	6.6	6.6	7.3	7.3	7.3
Tryptophan	1.8	1.8	1.8	2.0	2.0	2.0	2.2	2.2	2.2
Arginine	8.2	9.4	10.1	9.5	10.7	11.1	10.7	11.9	12.1
Isoleucine	5.4	5.9	6.1	6.2	6.7	6.7	6.9	7.4	7.3
Leucine	10.0	10.6	10.7	11.2	11.8	11.6	12.4	13.0	12.6
Valine	6.4	6.9	7.0	7.2	7.7	7.6	7.9	8.4	8.2

[1]Vitamin mineral premix (units per kilogram of feed): vitamin A, 8,000 IU; vitamin D_3, 3,000 IU; vitamin E, 25 IU; menadione, 1.5 mg; riboflavin, 5 mg; pantothenic acid, 8 mg; vitamin B_{12}, 0.012 mg; pyridoxine, 1.5 mg; thiamine, 1.5 mg; folic acid, 0.5 mg; niacin, 30 mg; biotin, 0.06 mg; iodine, 0.8 mg; copper, 10 mg; iron, 80 mg; selenium, 0.3 mg; manganese, 80 mg; zinc, 80 mg; calcium carbonate, 500 mg.
[2]Avizyme 1302 (Danisco Animal Nutrition, Marlborough, Wiltshire).

TABLE 7.4. Ingredient composition and calculated nutrient of experimental diets for feeding phase II (24 to 31 wk of age) based on 100 g/h/d feed intake

Ingredient (g/kg)	90% of NRC			NRC			110% of NRC		
Wheat	798.3	635.7	484.2	753.1	590.4	447.2	707.8	545.1	410.1
Pea	0.0	150.0	300.0	0.0	150.0	300.0	0.0	150.0	300.0
Soybean meal	66.7	69.0	60.8	107.1	109.7	93.7	147.8	150.3	126.5
Canola oil	16.4	28.8	39.9	21.1	33.5	43.6	25.8	38.2	47.4
Limestone	93.6	93.4	93.2	93.4	93.2	93.0	93.2	92.9	92.8
Dicalcium phosphate	10.5	10.6	10.7	10.5	10.6	10.7	10.5	10.6	10.7
Sodium chloride	2.6	2.8	3.0	2.7	2.9	3.0	2.8	3.0	3.1
L–Lysine HCL	3.1	1.4	0.0	2.8	1.1	0.0	2.6	0.9	0.0
DL–Methionine	1.1	1.1	1.2	1.4	1.5	1.6	1.8	1.8	2.1
L–Threonine	1.2	0.8	0.4	1.3	0.8	0.6	1.3	0.8	0.7
L–Tryptophan	0.0	0.0	0.1	0.0	0.5	0.1	0.0	0.0	0.1
Choline chloride	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin–mineral premix[1]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Enzyme[2]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Calculated nutrient (g/kg)									
AME (kcal/kg)	2,850	2,850	2,850	2,850	2,850	2,850	2,850	2,850	2,850
Crude protein (N × 6.25)	150.4	153.1	152.9	163.1	165.8	163.5	175.9	178.5	174.1
Calcium	38.0	38.0	38.0	38.0	38.0	38.0	38.0	38.0	38.0
Nonphytate phosphorous	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Linoleic acid	12.1	14.1	15.9	12.7	14.6	16.3	13.2	15.2	16.8
Lysine	7.0	7.0	7.0	7.8	7.8	7.8	8.6	8.6	8.6
Methionine	3.1	3.1	3.2	3.6	3.6	3.7	4.1	4.1	4.2
Met+Cys	5.9	5.9	5.9	6.6	6.6	6.6	7.2	7.2	7.2
Threonine	5.3	5.3	5.3	5.9	5.9	5.9	6.5	6.5	6.5
Tryptophan	1.6	1.6	1.6	1.8	1.8	1.8	2.0	2.0	2.0
Arginine	7.1	8.3	9.2	8.2	9.4	10.1	9.3	10.5	11.0
Isoleucine	4.8	5.3	5.6	5.4	5.9	6.1	6.1	6.6	6.7
Leucine	9.0	9.6	9.9	10.0	10.7	10.8	11.1	11.7	11.6
Valine	5.8	6.3	6.6	6.5	6.9	7.1	7.1	7.6	7.6

[1]Vitamin mineral premix (units per kilogram of feed): vitamin A, 8,000 IU; vitamin D_3, 3,000 IU; vitamin E, 25 IU; menadione, 1.5 mg; riboflavin, 5 mg; pantothenic acid, 8 mg; vitamin B_{12}, 0.012 mg; pyridoxine, 1.5 mg; thiamine, 1.5 mg; folic acid, 0.5 mg; niacin, 30 mg; biotin, 0.06 mg; iodine, 0.8 mg; copper, 10 mg; iron, 80 mg; selenium, 0.3 mg; manganese, 80 mg; zinc, 80 mg; calcium carbonate, 500 mg.
[2]Avizyme 1302 (Danisco Animal Nutrition, Marlborough, Wiltshire).

TABLE 7.5. Ingredient composition and calculated nutrient of experimental diets for feeding phase III (32 to 43 wk of age) based on 105 g/h/d feed intake

Ingredient (g/kg)	90% of NRC			NRC			110% of NRC		
Wheat	813.9	656.4	501.3	776.1	613.3	466.0	733.0	570.3	430.8
Pea	0.0	150.0	300.0	0.0	150.0	300.0	0.0	150.0	300.0
Soybean meal	54.2	51.8	47.1	87.9	90.4	78.3	126.5	129.0	109.5
Canola oil	14.5	26.3	37.8	18.4	30.8	41.4	22.8	35.2	44.9
Limestone	94.5	94.3	94.1	94.3	94.1	93.9	94.1	93.9	93.7
Dicalcium phosphate	8.7	8.8	8.9	8.7	8.8	8.9	8.7	8.8	8.9
Sodium chloride	2.6	2.8	2.9	2.6	2.8	3.0	2.7	2.9	3.1
L–Lysine HCL	3.0	1.5	0.0	2.9	1.2	0.0	2.7	1.0	0.0
DL–Methionine	0.9	1.0	1.0	1.3	1.3	1.4	1.6	1.6	1.8
L–Threonine	1.2	0.8	0.4	1.3	0.8	0.5	1.3	0.8	0.7
L–Tryptophan	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1
Choline chloride	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin–mineral premix[1]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Enzyme[2]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Calculated nutrient (g/kg)									
AME (kcal/kg)	2,850	2,850	2,850	2,850	2,850	2,850	2,850	2,850	2,850
Crude protein (N × 6.25)	146.6	147.9	148.7	157.3	160.0	158.7	169.4	172.1	168.8
Calcium	38.0	38.0	38.0	38.0	38.0	38.0	38.0	38.0	38.0
Nonphytate phosphorous	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
Linoleic acid	11.9	13.8	15.6	12.3	14.3	16.0	12.9	14.8	16.4
Lysine	6.7	6.7	6.7	7.4	7.4	7.4	8.2	8.2	8.2
Methionine	2.9	2.9	2.9	3.4	3.4	3.4	3.9	3.9	4.0
Met+Cys	5.6	5.6	5.6	6.2	6.2	6.2	6.9	6.9	6.9
Threonine	5.1	5.1	5.1	5.6	5.6	5.6	6.2	6.2	6.2
Tryptophan	1.6	1.5	1.5	1.7	1.7	1.7	1.9	1.9	1.9
Arginine	6.7	7.8	8.9	7.7	8.9	9.7	8.7	10.0	10.6
Isoleucine	4.6	5.0	5.4	5.1	5.6	5.9	5.7	6.3	6.4
Leucine	8.7	9.2	9.6	9.6	10.2	10.4	10.6	11.2	11.2
Valine	5.6	6.0	6.4	6.2	6.6	6.9	6.8	7.3	7.4

[1]Vitamin mineral premix (units per kilogram of feed): vitamin A, 8,000 IU; vitamin D_3, 3,000 IU; vitamin E, 25 IU; menadione, 1.5 mg; riboflavin, 5 mg; pantothenic acid, 8 mg; vitamin B_{12}, 0.012 mg; pyridoxine, 1.5 mg; thiamine, 1.5 mg; folic acid, 0.5 mg; niacin, 30 mg; biotin, 0.06 mg; iodine, 0.8 mg; copper, 10 mg; iron, 80 mg; selenium, 0.3 mg; manganese, 80 mg; zinc, 80 mg; calcium carbonate, 500 mg.
[2]Avizyme 1302 (Danisco Animal Nutrition, Marlborough, Wiltshire).

TABLE 7.6. Ingredient composition and calculated nutrient of experimental diets for feeding phase IV (43 to 56 wk of age) based on 110 g/h/d feed intake

Ingredient (g/kg)	90% of NRC			NRC			110% of NRC		
Wheat	828.5	689.7	525.5	797.2	634.5	483.4	755.9	593.1	449.6
Pea	0.0	150.0	300.0	0.0	150.0	300.0	0.0	150.0	300.0
Soybean meal	42.5	22.4	26.3	70.1	72.6	64.1	107.2	109.7	94.1
Canola oil	12.7	22.4	34.9	15.9	28.3	39.3	20.2	32.5	42.7
Limestone	95.3	95.2	94.9	95.2	94.9	94.7	95.0	94.7	94.6
Dicalcium phosphate	7.1	7.2	7.3	7.1	7.2	7.3	7.0	7.1	7.2
Sodium chloride	2.5	2.7	2.9	2.6	2.8	3.0	2.7	2.9	3.0
L–Lysine HCL	3.0	2.0	0.2	3.0	1.4	0.0	2.8	1.1	0.0
DL–Methionine	0.8	0.9	0.9	1.1	1.1	1.3	1.4	1.5	1.6
L–Threonine	1.1	0.9	0.4	1.2	0.8	0.5	1.3	0.8	0.6
L–Tryptophan	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.1
Choline chloride	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin–mineral premix[1]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Enzyme[2]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Calculated nutrient (g/kg)									
AME (kcal/kg)	2,850	2,850	2,850	2,850	2,850	2,850	2,850	2,850	2,850
Crude protein (N × 6.25)	143.0	139.5	142.5	152.0	154.7	154.3	163.6	166.3	164.0
Calcium	38.0	38.0	38.0	38.0	38.0	38.0	38.0	38.0	38.0
Nonphytate phosphorous	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3
Linoleic acid	11.7	13.3	15.3	12.0	14.0	15.8	12.5	14.5	16.2
Lysine	6.4	6.4	6.4	7.1	7.1	7.1	7.8	7.8	7.8
Methionine	2.7	2.7	2.7	3.1	3.2	3.2	3.6	3.6	3.7
Met+Cys	5.4	5.3	5.3	6.0	6.0	6.0	6.6	6.6	6.6
Threonine	4.8	4.8	4.8	5.4	5.4	5.4	5.9	5.9	5.9
Tryptophan	1.5	1.5	1.5	1.6	1.6	1.6	1.8	1.8	1.8
Arginine	6.4	7.0	8.3	7.2	8.4	9.3	8.2	9.4	10.2
Isoleucine	4.4	4.5	5.1	4.8	5.3	5.7	5.4	5.9	6.2
Leucine	8.4	8.4	9.0	9.1	9.7	10.0	10.1	10.7	10.8
Valine	5.4	5.5	6.0	5.9	6.4	6.6	6.5	7.0	7.1

[1]Vitamin mineral premix (units per kilogram of feed): vitamin A, 8,000 IU; vitamin D_3, 3,000 IU; vitamin E, 25 IU; menadione, 1.5 mg; riboflavin, 5 mg; pantothenic acid, 8 mg; vitamin B_{12}, 0.012 mg; pyridoxine, 1.5 mg; thiamine, 1.5 mg; folic acid, 0.5 mg; niacin, 30 mg; biotin, 0.06 mg; iodine, 0.8 mg; copper, 10 mg; iron, 80 mg; selenium, 0.3 mg; manganese, 80 mg; zinc, 80 mg; calcium carbonate, 500 mg.
[2]Avizyme 1302 (Danisco Animal Nutrition, Marlborough, Wiltshire).

TABLE 7.7. Effects of strain, pea inclusion, and lysine level on overall performance characteristics of laying hens

Parameter	Strain		Pea inclusion (g/kg)			Lysine intake (mg/h/d)			SEM[3]
	A[1]	B[2]	0	150	300	700	780	860	
Daily feed intake (g/hen)	99.5[b]	111.3[a]	105.0	105.1	106.2	105.9	105.1	105.3	0.68
Body weight at trial end (kg)	1.71	1.71	1.68[b]	1.71[a]	1.73[a]	1.66[c]	1.72[b]	1.75[a]	0.008
Body weight gain (kg)	0.409[a]	0.238[b]	0.297[b]	0.326[a]	0.348[a]	0.269[c]	0.330[b]	0.371[a]	0.012
Total hen housed egg production (%)	88.1[b]	92.4[a]	90.4	89.9	90.3	89.8	90.1	90.8	0.33
Total hen day egg production (%)	88.3[b]	93.6[a]	91.2	90.7	90.9	90.8	90.7	91.3	0.34
Egg weight (g)	57.5[b]	58.5[a]	57.5[b]	58.1[a]	58.5[a]	57.1[c]	58.2[b]	58.8[a]	0.140
Total feed to egg mass ratio (kg/kg)	1.97[b]	2.04[a]	2.01	2.00	2.00	2.05[a]	2.00[b]	1.97[c]	0.007
Total feed per dozen eggs (kg/dozen)	1.36[b]	1.43[a]	1.38	1.39	1.40	1.40	1.39	1.39	0.005
Egg–specific gravity	1.081[b]	1.084[a]	1.083	1.083	1.082	1.083	1.083	1.082	0.0002
Yolk color[4]	3.78[a]	3.30[b]	2.76[c]	3.71[b]	4.17[a]	3.43	3.52	3.67	0.073
Soft–shelled eggs[5] (%)	0.49	0.50	0.54	0.43	0.51	0.53	0.47	0.47	0.035
Cracked eggs[5] (%)	0.20[a]	0.13[b]	0.13	0.16	0.21	0.13	0.19	0.17	0.016
Broken eggs[5] (%)	0.38	0.33	0.27	0.40	0.39	0.32	0.41	0.33	0.027
Double yolked eggs[5] (%)	0.18[b]	0.31[a]	0.20	0.28	0.24	0.24	0.23	0.26	0.015
Abnormal eggs[5] (%)	0.06	0.06	0.08	0.05	0.05	0.04	0.08	0.05	0.008
Feather score[4]	18.33[a]	15.78[b]	17.23	17.14	16.82	16.55[b]	17.16[a]	17.51[a]	0.181
Mortality (%)[6]	0.59[b]	3.18[a]	2.22	1.56	1.84	2.67	1.78	1.15	0.353

[a–c] Means within the same row and main effects with different superscripts differ significantly ($P \leq 0.05$).
[1] Hy–Line CV–20.
[2] Lohmann LSL–Lite.
[3] SEM–Pooled standard error of the mean (N = 90).
[4] Determined at 54–wk of age.
[5] Determined for all egg production.
[6] Recorded daily (20 to 55 wk of age).

8.0. THE EFFECT OF DIETARY LEVEL OF FIELD PEA AND BALANCED AMINO ACIDS ON GROWTH PERFORMANCE OF BROILER CHICKENS

8.1. Abstract

Pea is an important crop in Western Canada with a considerable potential as a feed ingredient for broilers. Pea has also been recognized for its slowly degraded starch, which has been suggested to reduce the amino acid requirement of broilers. Therefore, an experiment was designed to investigate the maximum inclusion level of pea in broiler diets and the interaction with level of dietary amino acids. A growth trial with 3,480 broiler chickens was conducted as a 6 × 2 factorial arrangement evaluating 6 levels of pea inclusion (0, 150, 300, 450, 600, and 750 g/kg) and 2 levels of amino acids (100 and 85% of Ross × Ross 308 requirement). Each treatment was offered to five pens of 58 males from 0 to 35 d of age. No interaction was found between pea inclusion and amino acid level for all studied parameters. Pea inclusion level affected performance in an age dependent manner. Body weight gain (**BWG**) from 0 to 10, 10 to 25, and 25 to 35, and 0 to 35 d decreased when pea level exceeded 300, 600, 600, and 600 g/kg, respectively. Mortality corrected gain–to–feed ratio (**G:F**) was affected in a quadratic fashion by pea inclusion with the best efficiency at 150 and 450 g/kg for 0 to 10 and 10 to 25 d periods, respectively; G:F was unaffected by pea level from 25 to 35 and 0 to 35 d. The higher level of amino acids increased 0 to 35 d G:F ratio, and carcass and breast meat yield. Broilers fed pea levels above 450 g/kg had reduced carcass and breast weight as a proportion of live weight. In conclusion, maximum pea inclusion levels increased with broiler age, but the effect of slow digested starch from pea on amino acid requirement could not be confirmed.

Key words: pea, slow digested starch, amino acid, broiler chicken, performance

8.2. Introduction

Pea (*Pisum sativum* L.) has the potential to provide both dietary energy and protein in poultry diets (Castell et al., 1996), but uncertainty remains in regard to the nutritional value of this feedstuff and its upper level of inclusion in broiler diets. Field pea contains up to 490 g/kg (DM) starch (Wang and Daun, 2004), and in comparison to other starch sources, pea has a higher amylose to amylopectin ratio and most of the amylopectin chains are C–type (Daveby et al., 1998; Eliasson and Gudmundsson, 2006). In non–ruminant animals, amylose is less digested than amylopectin and C–type starch is more resistant to digestive enzymes than A–type starch. As a consequence, pea starch is slowly digested and often less well digested than starch from other cereal grains (Longstaff and McNab, 1987; Yutste et al., 1991; Daveby et al., 1998; Weurding et al., 2001; Meng and Slominski, 2005).

Starch is the main energy–yielding component of poultry diets and the AME value of feedstuffs is positively correlated with the extent of starch digestion (Wiseman et al., 2000). The rate and site of starch digestion are different among starch sources and the terms rapid digested (**RDS**), slow digested (**SDS**), and resistant starch (**RS**) have been introduced to characterize starch sources nutritionally (Englyst et al., 1992). Starches with different rates of digestion may have the same extent of starch digestion, but the site of starch digestion and glucose absorption in the small intestine sections are different.

These differences may affect the efficiency of starch utilization as well as other aspects of gastrointestinal function.

In diets with RDS, most starch digestion and glucose absorption occurs in the proximal portion of the small intestine, whereas other nutrients (e.g. amino acids) may not be absorbed at that section. The lack of nutrient absorption synchrony can affect the efficiency of their use for productive purposes (van den Borne et al., 2007). For instance, amino acids may be deaminated and catabolized for energy or used for gluconeogenesis in the absence of glucose. Moreover, insulin released in response to blood glucose stimulates protein deposition and inhibits gluconeogenesis (Björck, 2006). Finally, less glucose would be available to the distal part of small intestine, and more amino acids would be used as an energy source in that region. Use of available amino acids for energy in the small intestine would reduce its availability for protein deposition.

In human nutrition, the rate of starch digestion is related to blood glucose level. A relationship between glycemic index and a range of health benefits have been proposed as a result of consuming SDS (Jenkins et al., 1981). The rate of starch digestion in poultry diets has been reported to affect broiler growth performance with a mixture of rapidly and slowly degraded dietary starch improving broiler performance in contrast to diets containing exclusively rapidly degraded starch (Weurding et al., 2003a,b). Improved performance has been suggested to be due to the sparing of amino acids as a result of post–absorptive metabolic and hormonal effects and/or that the slowly degraded starch priovides energy for the distal part of the small intestine.

Much of the pea research with broilers has used winter–seeded pea cultivars and not spring–seeded pea cultivars. Spring–seeded, white–flowered pea cultivars, the

predominant type grown in Canada contain lower levels of anti–nutritional factors (**ANFs**) than winter–seeded varieties (Valdebouze et al. 1980; Brenes et al. 1993). It is probable that the level of ANFs would affect the pea inclusion limits in broiler diets. The inclusion of pea in broiler diets has been studied for many years. Much of this work has suggested 100 to 200 g/kg as the upper limit of pea inclusion (Moran et al., 1968; Castell et al., 1996; Igbasan and Guenter, 1996a,b; Fasina and Campbell, 1997; McNeill et al., 2004; Li et al., 2006; Gutierrez del Alamo et al., 2009; Nalle et al., 2010). On the other hand, Brenes et al. (1989) found no detrimental effect of pea inclusion up to 800 g/kg during (7 to 28 d). Brenes et al. (1993) found that feeding broilers 480 g/kg of pea resulted in similar growth performance to birds fed a corn–soybean diet. Farrell et al. (1999) found no effect on broiler growth rate during the starter and finisher phases when 200 or 300 g/kg pea were included in the diet. On the other hand, Cowieson et al. (2003) reported a reduction in broiler performance (21 d) fed 300 g/kg pea. McNeill et al. (2004) fed up to 200 g/kg pea in the diet and reported a decrease in body weight gain and feed intake but no effect on feed conversion ratio. They concluded that 100 g/kg of pea in broiler diets was the upper limit. Meng and Slominski (2005) reported that broiler performance (5 to 18 d) was reduced by feeding a corn–pea diet (300 g/kg of pea) compared with a corn–soybean meal diet. Moschini et al. (2005) and Diaz et al. (2006) found that overall broiler performance was not affected by inclusion of 350 g/kg of pea. Li et al. (2006) evaluated pea inclusion up to 500 g/kg in broiler starter diet (3 to 17 d) and reported similar performance for chicks fed 100 g/kg pea inclusion to maize–soybean diet; however, in another trial (0 to 40 d) the 300 g/kg of pea inclusion had no effect on broiler performance. Including 150 g/kg of pea in wheat–corn–soybean based broiler diet

(8 to 35 d) reduced FCR and increased feed intake (Czerwinski et al., 2010). The variable effect of pea inclusion in broiler diets might be due to different pea cultivars, different processing, or the use of an inaccurate pea nutrient profile in feed formulation.

Fewer studies have looked at the effect of pea inclusion on meat yield in broiler chickens. McNeill et al. (2004) reported no effect of pea inclusion up to 200 g/kg on breast weight as a proportion of live BW. Similarly, Moschini et al. (2005) found no effect of pea inclusion up to 350 g/kg on carcass, breast, and leg quarter cuts at 42 d of age. Diaz et al. (2005) found that carcass as a proportion of live BW was reduced by pea inclusion of 350 g/kg. Dehulled–micronized pea included at 400 g/kg had a positive effect on carcass traits of female broilers at 49 d of age (Laudadio and Tufarelli, 2010).

In vitro research in our laboratory has confirmed the slowly digested nature of pea starch compared with starch from barley, corn, and wheat (Ebsim et al., 2013). Based on these findings and the above literature, it was hypothesized that feeding slowly digested starch (SDS) from spring–seeded pea would improve broiler performance by sparing amino acid utilization. Therefore, the major objective of this experiment was to determine if the inclusion of pea (source of SDS) in broiler diets would reduce the bird's requirement for amino acids. A second objective was to study the effect of level of pea inclusion in the three growing phases, starter, grower, and finisher on broiler productivity.

8.3. Materials and Methods

The experimental protocol was approved by the Animal Care Committee of the University of Saskatchewan. All the experimental procedures were performed in accordance with the Guide to the Care and Use of Experimental Animals, Canadian Council on Animal Care (1993).

8.3.1. Experimental Design

A 6 × 2 factorial arrangement was used to determine the effects of level of dietary pea (0, 150, 300, 450, 600, and 750 g/kg), amino acids (85 and 100% of breed recommendation[1]), and their interaction on the performance and carcass quality of broiler chickens grown to 35 d of age. Broilers were housed in five environmentally independent rooms, each containing 12 pens. Dietary treatments were randomly assigned to pens within room, thereby yielding 5 replications per treatment. Room was considered a block from a statistical perspective. Pen was the experimental unit for all traits studied.

8.3.2. Birds and Housing

A total of 3,480 day–old–male chicks (Ross × Ross 308) were obtained from a commercial hatchery (Lilydale Inc., Wynyard, SK. Canada) and were housed in five experimental rooms. Pens in each room were bedded with wheat straw to a thickness of approximately 10 cm. Fifty–eight chicks were randomly assigned to each pen (200 × 230 cm) to provide a trial end density of 32 kg/m^2 based on chick placement numbers and anticipated growth rate. Each pen was provided with a tube feeder (137.5 cm circumference) and a drinker with 6 nipples (Lubing–4087). Birds had ad libitum access to water and feed throughout the trial. Room temperature was initially 34°C on d zero

[1] Broiler nutrition specification (June, 2007), as–hatched broilers 2.0 to 2.5 kg, Aviagen

and it was subsequently reduced in a gradual fashion to 22°C by d 28, where it was maintained for the remainder of the trial. The lighting period was 23L:1D during the first wk of the experiment with 20 lx of light intensity. At seven d of age, day length was reduced to18L:6D and light intensity to 10 lx.

8.3.3. Experimental Diets

With the exception of amino acids, diets were formulated to meet or exceed the Ross × Ross 308 recommendations (Broiler nutrition specification (June, 2007), as–hatched broilers 2.0 – 2.5 kg, Aviagen) for each growing phase. Pea (*Pisum sativum* L., cultivar CDC Golden) was included in the diets at 0, 150, 300, 450, 600, and approximately 750 g/kg and the level of amino acids was set at 85 and 100% of Aviagen recommendation. Starter, grower, and finisher diets were fed from d 0 to 10, 10 to 25, and 25 to 35, respectively. Nutritional balance across pea levels was maintained primarily by changing the levels of wheat, soybean meal (**SBM**), fat, and synthetic amino acids. Each level of amino acids diet was formulated to have at least the same level of digestible Met + Cys and Arg. Diets were formulated on a digestible amino acid basis as per Degussa (2005) for pea, wheat, and SBM. Pea replaced wheat and SBM and because of the higher content of lysine in pea, diets were formulated to maintain ratios of dietary essential amino acids to methionine content. Methionine was considered to be the most the limiting amino acid. Diets containing either wheat and SBM or pea and SBM, with a digestible amino acid content of 85 and 100% of breed recommendation, were formulated for the starter, grower and finisher phases. The diets containing intermediate levels of pea inclusion were calculated based on appropriate portions of the corresponding wheat and SBM, and pea and SBM diets. The starch digestion rate of the pea and wheat were based

on the results of in vitro starch digestion (Chapter 5.0). It was assumed that pea starch was digested slowly (65% at 2h) whereas wheat starch was rapidly digested (85 % at 2 h) (Weurding et al. 2001a.b). Diets were formulated to have approximately the same level of starch from different sources. Feed ingredients were ground before mixing using a hammer–mill (Model 170F8, Jacobson Machine Works, Minneapolis, Minn. 55427, USA) fitted with 6.35 mm screen–hole size and then steam pelleted using a California pellet–mill with a 4.75 mm die diameter. The preconditioning pelleting temperature range was 50 to 60°C. Starter and grower diets were fed in crumble form while finisher diets were provided in pellet form. The pea cultivar was CDC Golden and the wheat used in the experimental feeds was feed grade of unknown cultivar. The ingredient composition and calculated nutrient content of the starter, grower, and finisher diets are summarized in Table 8.1, 8.2, and 8.3, respectively. Feedstuffs and diets were analyzed for moisture using standard procedures (AOAC, 1990) and nitrogen content (crude protein = N × 6.25) was analyzed by a Leco–FP–528 Nitrogen Analyzer (Model 601–500–100, Serial # 3211, Leco Corporation, St. Joseph, MA, USA).

8.3.4. Data Collection

Body weight (**BW**) was measured on a pen basis at 0, 10, 25, and 35 d of age. Body weight gain (**BWG**) was calculated for 0 to 10, 10 to 25, 25 to 35, and 0 to 35 d. Feed intake (**FI**) was measured at 10, 25, and 35 d and gain–to–feed ratio (**G:F**) was determined based on BWG and FI for each growing phase and for the overall experiment. The G:F calculation was adjusted for mortality using the BW of dead and cull birds. Mortality was recorded daily and dead birds were collected and weighed individually.

A subjective scoring system was used to assess litter condition for three consistent areas in each pen (at the nipple drinker, feeder, and pen door). Litter quality was classified into three categories, 1) dry with no packing or fecal build–up, 2) some non–continuous fecal build–up (< 50% of monitored area), and 3) wet and mostly covered by fecal built–up (> 50% of monitored area). The quality of litter was assessed at three times over the experimental period, 15, 22 and 31 d of age.

At the end of the experiment, 6 birds were randomly selected from each pen and double wing–banded for meat yield determination (30 birds per treatment). Birds were individually weighed after feed (4 h) and water (additional 2 h) withdrawal prior to loading on slaughter day. Birds were slaughtered within 10 hours of the initiation of feed withdrawal. After slaughtering in a commercial processing plant (Lilydale Inc., Wynyard, SK. Canada), birds were packed in ice and returned to the University of Saskatchewan for carcass evaluation. Carcasses were weighed and then separated into the following components: breast (skin, Pectoralis major and Pectoralis minor), left drum (skin, meat, bone), left thigh (skin, meat, bone), intact right drumstick, intact right thigh, wings, abdominal fat pad, and back/rack. Component weights are presented as a percentage of live body weight. Because broilers were slaughtered in a commercial processing plant, the amount of abdominal fat remaining on the carcass was variable. Therefore abdominal fat is given for completeness, but the values do not reflect experimental treatments accurately and were not used in the interpretation of results.

8.4. Statistical Analysis

Data were analyzed as a randomized complete block design (RCBD) in a two–way factorial arrangement (2 × 6) with the main effects of two levels of amino acid (85 and 100% of breed recommendation) and six levels of pea inclusion (0, 150, 300, 450, 600, and 750 g/kg). The normality of data was checked prior to analysis using the PROC Univariate test of SAS Institute (2008). Mortality, litter condition, and meat yield data were converted to a percentage and transformed (log+1) prior to ANOVA analyses. All data were subjected to analysis of variance using the PROC Mixed procedure of SAS Institute (2008). Tukey's Studentized Range Test was used for mean separation and pdmix800 was used to provide letters for differences (Saxton, 1998). The relationships between the dependant variable and level of pea and amino acid inclusion were studied using PROC REG (Regression) and PROC REGRS (Response Surface Regression) of SAS. All dependent variable data are presented as means and pooled SEM. Differences were considered significant if the probability of difference was less than or equal to 0.05, unless otherwise stated.

8.5. Results

The effect of blocks (rooms) was not significant and therefore the experimental model was adjusted to remove blocks. No interactions were found between diet level of pea and amino acids for any response criteria and for any time period. Therefore, the experimental results are presented as main effects for pea and amino acid inclusion levels.

8.5.1. Starter Phase (0 to 10 d)

Performance of broiler chickens from d 0 to 10 is presented in Table 8.4. Average feed intake (FI), BW, body weight gain (BWG), gain–to–feed ratio (G:F), and incidence of mortality were not affected by the level of amino acid intake. However, pea inclusion level affected the FI, BW, BWG, and G:F. Pea inclusion had a quadratic effect on all performance parameters and the overall relation to pea inclusion was described by the following equations:

FI – $Y = 0.274171 + 0.000042023\ X - 0.000006614\ X^2$

BW (10 d) – $Y = 0.27336 + 0.000131\ X - 0.000008526\ X^2$

BWG – $Y = 0.228497 + 0.000128\ X - 0.000008463\ X^2$

G:F – $Y = 0.840548 + 0.000338\ X - 0.000012464\ X^2$.

Based on Tukey's mean separation test, feeding pea at levels higher than 300 g/kg diet resulted in a lower FI, BW, and BWG, whereas, G:F was only depressed with the pea inclusion above 600 g/kg ($P < 0.01$). Death loss was affected by level of pea but regression analysis failed to demonstrate a relationship.

8.5.2. Grower Phase (10 to 25 d)

In the growing phase (Table 8.5), feeding the 85% of digestible amino acid level had no effect on FI, BW, BWG, G:F, and mortality compared to the 100% recommendation for the Ross × Ross 308 genotype. Diet pea level affected BW, BWG and G:F in a quadratic fashion, while FI decreased in a linear fashion with increasing pea inclusion. The curvilinear relationship between pea inclusion and BW, BWG and G:F are described by the quadratic equations:

BW (25 d) – $Y = 1.242127 + 0.000866\ X - 0.000029039\ X^2$

BWG – $Y = 0.968766 + 0.000735\ X - 0.000020513\ X^2$

G:F – $Y = 0.637278 + 0.000886\ X - 0.000009919\ X^2$

The linear equation for the effect of pea level on FI is $Y = 1.55552 - 0.00164\ X$.

Using mean separation to establish the response to dietary pea level, FI was lower when the pea inclusion level exceeded 450g/kg diet. However, BW and BWG were only lower in birds fed 750 g pea /kg diet and G:F was highest at a pea inclusion level of 450 g/kg diet.

8.5.3. Finisher Phase (25 to 35 d)

Broiler chicken performance in the finisher phase is presented in Table 8.6. Level of diet amino acids did not affect any response criteria. Dietary pea level did not affect FI, G:F, or mortality. However, BW was affected in a quadratic pattern by pea level in the diet, while BWG decreased in a linear fashion with increasing level of pea. The respective equations are as follows:

BW (35 d) – $Y = 2.241422 + 0.000801\ X - 0.000037262\ X^2$

BWG – $Y = 1.00546 - 0.00068203\ X$

Mean separation indicated that only the highest level of pea inclusion (750 g/kg) reduced BW.

8.5.4. Overall Performance (0 to 35 d)

Growth performance of broiler chickens over the whole experimental period is given in Table 8.7. Only G:F was affected by the level of amino acids. The higher amino acid level increased G:F compared with the lower level (1.659 vs. 1.634; respectively). Pea inclusion affected BW (35 d – described above) and BWG in a quadratic fashion.

Feed intake and G:F decreased in a linear fashion as the pea inclusion increased. The equations for the latter response are:

BWG – $Y = 2.196559 + 0.000797\ X - 0.000037199\ X^2$

FI – $Y = 3.76539 - 0.00235\ X$

G:F – $Y = 0.61492 - 0.0018919\ X$

Only the highest level of pea inclusion (750 g/kg) decreased FI, BW, and BWG significantly. Neither dietary pea level nor amino acid affected overall mortality and the condition of the litter quality as it was assessed at the end of trial.

8.5.5. Meat Yield

The results of meat yield as percentage of live body weight are presented in Table 8.8. Only Pectoralis major and total breast meat were affected by the level of amino acids with lower yields for the lower level of amino acids. Level of dietary pea affected carcass, pectoral major, total breast, breast skin, abdominal fat, drum meat, and drum skin weight as a percentage of live weight. However, there were no differences among levels of pea inclusion up to 450 g/kg on proportional carcass, pectoral major, and total breast weights.

Carcass, Pectoralis major, total breast, and abdominal fat weight were affected by pea inclusion in a curvilinear fashion as shown in the following equations:

Carcass weight – $Y = 68.735492 + 0.000192\ X - 0.00042\ X^2$

Pectoralis major – $Y = 16.036789 + 0.008749\ X - 0.000402\ X^2$

Total breast – $Y = 19.532628 + 0.00663\ X - 0.000394\ X^2$

Abdominal fat – $Y = 0.697878 + 0.003307\ X - 0.000063758\ X^2$

Skin breast (linear) – $Y = 1.81914 - 0.00382\ X$

8.6. Discussion

A hypothesis of this research was that feeding a SDS from pea would spare amino acids in broiler chickens, as it had previously been shown to improve amino acid utilization (Weurding et al., 2003). The two levels of amino acids used in this research were based on broiler requirement recommended by Aviagen and the 85% treatment was assumed to be deficient, thereby permitting the amino acid sparing effect of SDS to be seen. However, amino acid level only affected G:F (0 to 35 d of age), and carcass and breast meat yield as a proportion of live BW, where the 100% level improved all three parameters. This result indicates that the 85% level of amino acids may not have been sufficiently deficient to permit confirmation of an effect of SDS. The lack of interactions between dietary levels of pea (SDS levels) and amino acids also does not support this hypothesis. Based on these two factors, the sparing effect of SDS on amino acids cannot be confirmed from this research.

Feeding pea up to 300 g/kg had no effect on BW, BWG, and FI during the starter phase (0 to 10 d). These results are in a good agreement with results of previous research (Perez–Maldonado et al., 1998; Farrell et al., 1999; Diaz et al., 2006; Nalle et al. 2010), but are in contrast to (Igbasan and Guenter, 1996b; Fasina and Campbell, 1997; Cowieson et al., 2003; McNeill et al., 2004; and Li et al., 2006). Although the exact reason for differences in research results could not be identified, a number of factors may be involved including pea cultivar, chicken breed, age at which pea was fed, feed formulation, and feed processing. The reduction in bird performance with pea inclusion above 300 g/kg diet may be related to the young bird's capacity to digest pea nutrients. It is recognized that young birds have limited digestive capacity (Uni et al., 1999; Sklan and

Noy, 2000; Sklan, 2001). In contrast to our study, broiler performance from 0 to 15 d was unaffected by feeding a diet with 700 g/kg of dehulled pea (Daveby et al., 1998). This result suggests that hull fibre may negatively affect broiler performance. Also feed form might have some impact on broiler performance as the FI was affected in the same pattern as BW and BWG. Our data suggest that the maximum level of pea inclusion during the starter phase should be no higher than 300 g/kg.

During the grower phase from 10 to 25 d, chickens were able utilize up to 600 g/kg of pea with no detrimental effect on performance, and feeding pea diets resulted in better G:F compared with the wheat soybean control diet. The best G:F was achieved at the 450 g/kg of pea inclusion. In contrast to Brenes et al. (1989), FI was decreased in a linear fashion and G:F had a quadratic relation to pea inclusion. However, comparing our results to Brenes et al. (1989) may not be appropriate because their diets contained a wide range of oil (0 to 105 g/kg). In another experiment, Brenes et al. (1993) found that feeding broiler 480 g/kg of pea had similar performance to birds fed a corn–soybean diet.

A complete replacement of wheat and SBM with pea in finisher diets had no effect on FI, BWG, and G:F. Only the BW was affected by the higher level of pea inclusion because these birds grew more slowly during starter and grower phases. Previous research has shown that including 350 g/kg of pea (raw or extruded) in broiler diets fed from 29 to 42 d of age had no adverse effect on broiler performance compared to corn soybean meal diets (Diaz et al., 2006). Moreover, using dehulled–micronized pea at 400 g/kg in female broiler diets during 14 to 49 d of age had a positive effect on broiler growth performance as well as carcass traits (Laudadio and Tufarelli, 2010). In contrast to our results, Gutierrez del Alamo et al. (2009) found that feeding a wheat–pea diet from

21 to 34 d with 350 g/kg pea decreased performance in comparison to diets with 0 or 170 g/kg of pea.

Data for the entire trial (0 to 35 d) indicated that birds performed was similarly with pea inclusion up to 600 g/kg. However, it should be taken into account that the performance during starter, grower, and finisher phases affected overall performance. The quadratic effect of pea inclusion on BW and BWG was observed as well as the linear effect on FI. Feeding 600 g/kg of pea inclusion had no adverse effect on bird performance. These results are in agreement with the findings of Brenes et al. (1989) and Meng and Slominski (2005).

In contrast to the current research, Perez–Maldonado et al. (1998) recommended 300 g/kg as the upper level of pea inclusion in starter and finisher diets. Whereas Diaz et al. (2006) found 350 g/kg was optimum level of pea inclusion. Gutierrez et al. (2009) found that feeding wheat–pea diet (170 g/kg pea) had better performance in compared with 350 g/kg pea fed from 0 to 34 d. Also, Czerwinski (2010) reported that feeding 150 g/kg of pea increased FI and decreased G:F compared with wheat and maize diets (8 d to 35). Our data are in good agreement with previous studies as carcass, pectoral major, and total breast weight as a proportional of live BW were not affected by pea inclusion up to 450 g/kg (McNeill et al., 2004; Diaz et al., 2005; Moschini et al., 2005; Laudadio and Tufarelli, 2010). It should be noted that birds in this experiment were grown for 35 d whereas in other experiments broilers were grown to 42 and 49 d of age.

In conclusion, results from this experiment are supportive of the utilization of pea in broiler diets as a substitution for SBM and wheat. The data of this experiment confirm that pea is a suitable feedstuff for broiler chickens. The effect of pea inclusion was age

related, with older birds performing better at higher levels of pea inclusion in the diet. Moreover, the lack of relationship between pea inclusion and amino acid intake indicates a limited contribution from the SDS of pea to the amino acid utilization. The results from this experiment support the inclusion pea of up to 300, 600, 750 g/kg for broiler starter, grower, and finisher periods, respectively.

TABLE 8.1. Composition and calculated nutrient content of starter diets (%) fed from 0 to 10 d of age

Feed ingredients	Amino acid levels based on 85% of breed recommendation[1]						Amino acid levels based on 100% breed recommendation[1]					
Pea	–	15.62	31.24	46.85	62.47	78.09	–	15.33	30.66	45.98	61.31	76.64
Wheat	55.22	44.18	33.13	22.09	11.04	–	55.75	44.60	33.45	22.30	11.15	–
Soybean meal	36.41	30.98	25.54	20.11	14.67	9.24	35.58	30.56	25.55	20.53	15.52	10.50
Canola oil	3.76	4.52	5.29	6.05	6.82	7.58	3.60	4.38	5.16	5.95	6.73	7.51
Dicalcium phosphate	1.86	1.88	1.90	1.91	1.93	1.95	1.87	1.89	1.90	1.92	1.93	1.95
Limestone	1.39	1.39	1.39	1.39	1.39	1.39	1.40	1.40	1.39	1.39	1.38	1.38
Sodium chloride	0.49	0.49	0.49	0.50	0.50	0.50	0.49	0.49	0.49	0.50	0.50	0.50
L–Lysine HCl	–	–	–	–	–	–	0.20	0.16	0.12	0.08	0.04	–
DL–Methionine	0.14	0.20	0.26	0.31	0.37	0.43	0.29	0.34	0.40	0.45	0.51	0.56
L–Threonine	–	0.02	0.03	0.05	0.06	0.08	0.09	0.11	0.13	0.15	0.17	0.19
L–Tryptophan	–	–	–	0.01	0.01	0.01	–	0.01	0.02	0.02	0.03	0.04
Vitamin–mineral premix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Avizyme 1302[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Coccistac[4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Stafac[5]	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Calculated nutrient content												
Crude protein (N × 6.25)	25.00	24.40	23.80	23.20	22.60	22.00	25.00	24.49	23.99	23.48	22.98	22.47
Crude fat	5.47	6.09	6.72	7.34	7.97	8.59	5.31	5.95	6.59	7.23	7.87	8.51
Calcium	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05
Nonphytate phosphorus	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Linoleic acid	1.31	1.47	1.62	1.78	1.93	2.09	1.27	1.43	1.59	1.76	1.92	2.08
M.E (kcal/kg)	3000	3000	3000	3000	3000	3000	3000	3000	3000	3000	3000	3000
Digestible Met + Cys	0.80	0.80	0.80	0.80	0.80	0.80	0.94	0.94	0.94	0.94	0.94	0.94
Digestible Lysine	1.14	1.16	1.18	1.21	1.23	1.25	1.27	1.27	1.27	1.27	1.27	1.27
Digestible Tryptophan	0.29	0.27	0.24	0.22	0.19	0.17	0.28	0.26	0.25	0.23	0.22	0.20
Digestible Threonine	0.76	0.75	0.74	0.73	0.72	0.71	0.83	0.83	0.83	0.83	0.83	0.83

[1]Broiler nutrition specification (June, 2007), as–hatched broilers 2.0 – 2.5 kg, www.aviagen.com.
[2]Supplied per kilogram of diet: vitamin A, 9425 IU (retinyl acetate + retinyl palmitate); vitamin D, 3055 IU; vitamin E, 50 IU (DL–α–tocopheryl acetate); vitamin K, 1.43 mg; thiamine, 1.95 mg; riboflavin, 6.5 mg; niacin, 65 mg; pyridoxine, 3.25 mg; vitamin B_{12}, 0.013 mg; pantothenic acid,13.0 mg; folic acid, 1.1 mg; biotin, 0.163 mg; antioxidant, 0.081 mg; iron, 55 mg; zinc, 60.5 mg; manganese, 74 mg; copper, 5.5 mg; iodine, 0.72 mg; and selenium, 0.3 mg.
[3]Avizyme 1302, Danisco Animal Nutrition, Marlborough, Wiltshire, UK.
[4]Coccistac, Phibro Animal Health, Ridgefield Park, NJ, USA.
[5]Stafac–44, Phibro Animal Health, Ridgefield Park, NJ, USA.

TABLE 8.2. Composition and calculated nutrient content of grower diets (%) fed from 10 to 25 d of age

Feed ingredients	Amino acid levels based on 85% breed recommendation[1]						Amino acid levels based on 100% breed recommendation[1]					
Pea	–	16.31	32.63	48.94	65.26	81.57	–	14.86	29.71	44.57	59.42	74.28
Wheat	62.30	49.84	37.38	24.92	12.46	0.00	61.75	49.40	37.05	24.70	12.35	0.00
Soybean meal	29.83	25.02	20.21	15.40	10.59	5.78	30.09	26.65	23.21	19.77	16.33	12.89
Canola oil	3.77	4.65	5.53	6.41	7.29	8.17	3.72	4.61	5.51	6.40	7.30	8.19
Dicalcium phosphate	1.62	1.64	1.65	1.67	1.68	1.70	1.62	1.63	1.64	1.65	1.66	1.67
Limestone	1.17	1.17	1.16	1.16	1.15	1.15	1.17	1.16	1.15	1.15	1.14	1.13
Sodium chloride	0.48	0.48	0.49	0.49	0.50	0.50	0.48	0.48	0.49	0.49	0.50	0.50
L–Lysine HCl	–	–	–	–	–	–	0.15	0.12	0.09	0.06	0.03	–
DL–Methionine	0.10	0.15	0.21	0.26	0.32	0.37	0.23	0.27	0.31	0.36	0.40	0.44
L–Threonine	–	–	0.01	0.01	0.02	0.02	0.06	0.08	0.10	0.12	0.14	0.16
L–Tryptophan	–	–	–	0.01	0.01	0.01	–	–	–	0.01	0.01	0.01
Vitamin–mineral premix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Avizyme 1302[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Coccistac[4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Stafac[5]	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.03	0.03	0.03	0.03	0.025
Calculated nutrient content												
Crude protein (N × 6.25)	22.69	22.35	22.01	21.68	21.34	21.00	23.00	23.00	23.00	23.00	23.00	23.00
Crude fat	5.59	6.31	7.03	7.75	8.47	9.19	5.53	6.26	6.99	7.72	8.45	9.18
Calcium	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Nonphytate phosphorus	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Linoleic acid	1.32	1.50	1.68	1.87	2.05	2.23	1.31	1.49	1.68	1.86	2.05	2.23
M.E (kcal/kg)	3050	3050	3050	3050	3050	3050	3050	3050	3050	3050	3050	3050
Digestible Met + Cys	0.71	0.71	0.71	0.71	0.71	0.71	0.84	0.84	0.84	0.84	0.84	0.84
Digestible Lysine	0.98	1.03	1.07	1.12	1.16	1.21	1.10	1.14	1.18	1.22	1.26	1.30
Digestible Tryptophan	0.26	0.24	0.22	0.19	0.17	0.15	0.26	0.24	0.23	0.21	0.20	0.18
Digestible Threonine	0.67	0.66	0.65	0.64	0.63	0.62	0.73	0.75	0.77	0.79	0.81	0.83

[1]Broiler nutrition specification (June, 2007), as–hatched broilers 2.0 – 2.5 kg, www.aviagen.com.
[2]Supplied per kilogram of diet: vitamin A, 9425 IU (retinyl acetate + retinyl palmitate); vitamin D, 3055 IU; vitamin E, 50 IU (DL–α–tocopheryl acetate); vitamin K, 1.43 mg; thiamine, 1.95 mg; riboflavin, 6.5 mg; niacin, 65 mg; pyridoxine, 3.25 mg; vitamin B_{12}, 0.013 mg; pantothenic acid,13.0 mg; folic acid, 1.1 mg; biotin, 0.163 mg; antioxidant, 0.081 mg; iron, 55 mg; zinc, 60.5 mg; manganese, 74 mg; copper, 5.5 mg; iodine, 0.72 mg; and selenium, 0.3 mg.
[3]Avizyme 1302, Danisco Animal Nutrition, Marlborough, Wiltshire, UK.
[4]Coccistac, Phibro Animal Health, Ridgefield Park, NJ, USA.
[5]Stafac–44, Phibro Animal Health, Ridgefield Park, NJ, USA.

TABLE 8.3. Composition and calculated nutrient content of finisher diets (%) fed from 25 to 35 d of age

Feed ingredients	Amino acid levels based on 85% breed recommendation[1]						Amino acid levels based on 100% breed recommendation[1]					
Pea	–	16.45	32.90	49.34	65.79	82.24	–	15.57	31.14	46.70	62.27	77.84
Wheat	61.93	49.54	37.16	24.77	12.39	–	62.72	50.18	37.63	25.09	12.54	–
Soybean meal	30.64	25.63	20.62	15.60	10.59	5.58	29.80	25.82	21.84	17.87	13.89	9.91
Canola oil	3.70	4.58	5.45	6.33	7.20	8.08	3.60	4.50	5.40	6.29	7.19	8.09
Dicalcium phosphate	1.46	1.47	1.49	1.50	1.52	1.53	1.46	1.47	1.48	1.50	1.51	1.52
Limestone	1.13	1.13	1.12	1.12	1.11	1.11	1.13	1.12	1.12	1.11	1.11	1.10
Sodium chloride	0.38	0.39	0.40	0.40	0.41	0.42	0.41	0.41	0.41	0.42	0.42	0.42
DL–Methionine	0.03	0.09	0.14	0.20	0.25	0.31	0.15	0.20	0.24	0.29	0.33	0.38
L–Threonine	–	–	–	–	–	–	–	–	–	0.01	0.01	0.01
Vitamin–mineral premix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Avizyme 1302[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Coccistac[4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Stafac[5]	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.03	0.03	0.03	0.03	0.025
Calculated nutrient content												
Crude protein (N × 6.25)	23.00	22.60	22.20	21.79	21.39	20.99	22.76	22.64	22.53	22.41	22.30	22.18
Crude fat	5.52	6.24	6.95	7.67	8.38	9.10	5.43	6.16	6.90	7.63	8.37	9.10
Calcium	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Nonphytate phosphorus	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Linoleic acid	1.31	1.49	1.67	1.85	2.03	2.21	1.29	1.47	1.66	1.84	2.03	2.21
M.E (kcal/kg)	3050	3050	3050	3050	3050	3050	3050	3050	3050	3050	3050	3050
Digestible Met + Cys	0.65	0.65	0.65	0.65	0.65	0.65	0.76	0.76	0.76	0.76	0.76	0.76
Digestible Lysine	1.00	1.04	1.08	1.13	1.17	1.21	0.98	1.04	1.10	1.15	1.21	1.27
Digestible Tryptophan	0.26	0.24	0.21	0.19	0.16	0.14	0.26	0.24	0.22	0.20	0.18	0.16
Digestible Threonine	0.68	0.66	0.65	0.63	0.62	0.60	0.67	0.67	0.66	0.66	0.65	0.65

[1]Broiler nutrition specification (June, 2007), as–hatched broilers 2.0 – 2.5 kg, www.aviagen.com.
[2]Supplied per kilogram of diet: vitamin A, 9425 IU (retinyl acetate + retinyl palmitate); vitamin D, 3055 IU; vitamin E, 50 IU (DL–α–tocopheryl acetate); vitamin K, 1.43 mg; thiamine, 1.95 mg; riboflavin, 6.5 mg; niacin, 65 mg; pyridoxine, 3.25 mg; vitamin B_{12}, 0.013 mg; pantothenic acid,13.0 mg; folic acid, 1.1 mg; biotin, 0.163 mg; antioxidant, 0.081 mg; iron, 55 mg; zinc, 60.5 mg; manganese, 74 mg; copper, 5.5 mg; iodine, 0.72 mg; and selenium, 0.3 mg.
[3]Avizyme 1302, Danisco Animal Nutrition, Marlborough, Wiltshire, UK.
[4]Coccistac, Phibro Animal Health, Ridgefield Park, NJ, USA.
[5]Stafac–44, Phibro Animal Health, Ridgefield Park, NJ, USA.

TABLE 8.4. Effect of level of diet pea and amino acid levels on broiler growth performance from 0 to 10 d (starter diets)

Performance parameters	Pea level (g/kg)							Amino acid level			SEM[1]
	0	150	300	450	600	750	*P* value	0.85	1.00	*P* value	
Body weight – 10 d (kg)**	0.273[a]	0.275[a]	0.267[ab]	0.262[bc]	0.253[c]	0.234[d]	< 0.001	0.261	0.261	NS	0.0020
Body weight gain (kg)**	0.228[a]	0.230[a]	0.222[ab]	0.217[bc]	0.208[c]	0.189[d]	< 0.001	0.216	0.216	NS	0.0020
Feed intake (kg/bird)**	0.274[a]	0.274[a]	0.269[ab]	0.262[bc]	0.254[c]	0.240[d]	< 0.001	0.261	0.263	NS	0.0019
Gain : Feed (g/g)**	0.840[a]	0.848[a]	0.831[a]	0.833[a]	0.820[ab]	0.793[b]	< 0.001	0.829	0.826	NS	0.0036
Mortality (%)	2.07[ab]	3.79[a]	2.07[ab]	1.38[b]	1.38[b]	2.59[ab]	0.021	2.07	2.36	NS	0.2428

[1]SEM = Standard error of the mean.
[a-d] Means in the same row (within dietary treatments) with common superscripts do not differ significantly ($P < 0.05$).
** = Quadratic regression with ($P < 0.05$).

TABLE 8.5. Effect of pea inclusion and dietary amino acid levels on broiler performance from 10 to 25 d (grower diets)

Performance parameters	Pea inclusion (g/kg)							Amino acid level			SEM[1]
	0	150	300	450	600	750	*P* value	0.85	1.00	*P* value	
Body weight – 25 d (kg)**	1.245[a]	1.248[a]	1.233[a]	1.220[a]	1.207[a]	1.135[b]	< 0.001	1.207	1.222	NS	0.0066
Body weight gain (kg)**	0.972[a]	0.974[a]	0.965[a]	0.958[a]	0.954[a]	0.901[b]	< 0.001	0.947	0.961	NS	0.0049
Feed intake (kg / bird)*	1.553[a]	1.527[ab]	1.513[ab]	1.477[bc]	1.474[bc]	1.420[c]	< 0.001	1.492	1.497	NS	0.0088
Gain to Feed (g/g)**	0.639[b]	0.647[ab]	0.651[ab]	0.662[a]	0.654[ab]	0.647[ab]	0.031	0.646	0.654	NS	0.0022
Mortality (%)	1.62	1.73	1.63	1.69	1.13	1.64	NS	1.57	1.58	NS	0.074

[1]SEM = Standard error of the mean.
[a-d] Means in the same row (within dietary treatments) with common superscripts do not differ significantly ($P < 0.05$).
* = Linear regression with ($P < 0.05$).
** = Quadratic regression with ($P < 0.05$).

TABLE 8.6. Effect of pea inclusion and dietary amino acid levels on broiler performance from 25 to 35 d (finisher diets)

Performance parameters	Pea inclusion (g/kg)							Amino acid level			SEM[1]
	0	150	300	450	600	750	*P* value	0.85	1.00	*P* value	
Body weight – 35 d (kg)**	2.247[a]	2.238[a]	2.225[a]	2.203[a]	2.170[ab]	2.083[b]	0.003	2.186	2.203	NS	0.0121
Body weight gain (kg)*	1.002	0.990	0.993	0.983	0.964	0.948	NS	0.979	0.981	NS	0.0075
Feed intake (kg/bird)	1.842	1.810	1.858	1.844	1.824	1.824	NS	1.850	1.817	NS	0.0077
Gain to Feed (g/g)	0.546	0.549	0.538	0.539	0.532	0.524	NS	0.533	0.543	NS	0.0038
Mortality (%)	3.79	4.31	3.10	3.62	2.59	4.66	NS	3.91	3.45	NS	0.327

[1]SEM = Standard error of the mean.
[a-c] Means in the same row (within dietary treatments) with common superscripts do not differ significantly ($P < 0.05$).
* = Linear regression with ($P < 0.05$).
** = Quadratic regression with ($P < 0.05$).

TABLE 8.7. Effect of pea inclusion and dietary amino acid levels on broiler performance from 0 to 35 d (overall)

Performance parameters	Pea inclusion (g/kg)							Amino acid level			SEM[1]
	0	150	300	450	600	750	*P* value	0.85	1.00	*P* value	
Body weight - 35 d(kg)**	2.247[a]	2.238[a]	2.225[a]	2.203[a]	2.170[ab]	2.083[b]	0.003	2.186	2.203	NS	0.0121
Body weight gain (kg)**	2.202[a]	2.193[a]	2.180[a]	2.158[a]	2.125[ab]	2.038[b]	0.003	2.141	2.158	NS	0.0121
Feed intake (kg/bird)*	3.761[a]	3.715[ab]	3.721[ab]	3.669[ab]	3.609[ab]	3.588[b]	0.009	3.696	3.659	NS	0.0164
Gain to Feed (g/g)*	0.609	0.616	0.608	0.613	0.605	0.595	NS	0.603[a]	0.612[b]	0.045	0.0022
Mortality (%)	10.17	13.10	10.00	9.83	7.07	12.41	NS	10.58	10.29	NS	0.5685
Litter quality[2]	1.83	1.78	1.58	1.55	1.78	1.43	NS	1.73	1.58	NS	0.06

[1]SEM = Standard error of the mean.
[2]Litter quality was classified into three categories, 1) dry with no packing or fecal built–up, 2) some non–continuous fecal build–up (< 50% of monitored area), and 3) wet and mostly covered by fecal built–up (> 50% of monitored area).
[a, b] Means in the same row (within dietary treatments) with common superscripts do not differ significantly ($P < 0.05$).
* = Linear regression with ($P < 0.05$).
** = Quadratic regression with ($P < 0.05$).

TABLE 8.8. Effect of pea inclusion and dietary amino acid levels on broiler meat yield as a percentage of live body weight at 35 d of age

	Pea inclusion (g/kg)							Amino acid level			SEM[1]
	0	150	300	450	600	750	*P* value	0.85	1.00	*P* value	
Carcass**	68.91[a]	68.32[a]	68.19[a]	68.27[a]	67.12[b]	66.41[b]	< 0.001	67.80	67.90	NS	0.110
Pectoral major**	16.02[a]	16.12[a]	15.72[a]	15.81[a]	14.98[b]	14.47[b]	< 0.001	15.35[b]	15.66[a]	0.011	0.069
Pectoral minor	3.51	3.47	3.37	3.46	3.42	3.38	NS	3.44	3.43	NS	0.020
Total breast**	19.53[a]	19.59[a]	19.09[a]	19.26[a]	18.40[b]	17.85[b]	< 0.001	18.79[b]	19.09[a]	0.027	0.076
Breast skin*	1.80[a]	1.71[ab]	1.73[ab]	1.73[ab]	1.59[bc]	1.49[c]	< 0.001	1.69	1.66	NS	0.018
Abdominal fat**	0.72[a]	0.70[ab]	0.74[a]	0.73[a]	0.71[ab]	0.56[b]	0.011	0.72	0.66	NS	0.016
R –Thigh whole	6.20	6.13	6.15	6.19	6.30	6.09	NS	6.19	6.16	NS	0.023
L –Thigh meat	4.56	4.45	4.53	4.53	4.49	4.41	NS	4.47	4.51	NS	0.026
L –Thigh skin	0.81	0.81	0.79	0.79	0.79	0.71	NS	0.79	0.78	NS	0.012
L –Thigh bone	0.84	0.82	0.85	0.85	0.88	0.87	NS	0.86	0.85	NS	0.006
R –Drum whole	4.85	4.91	4.88	4.93	4.85	4.87	NS	4.90	4.86	NS	0.015
L –Drum meat	3.15[b]	3.22[ab]	3.15[b]	3.28[a]	3.15[b]	3.16[ab]	0.010	3.20	3.16	NS	0.013
L –Drum skin	0.53[ab]	0.55[a]	0.51[ab]	0.51[ab]	0.51[ab]	0.49[b]	0.034	0.52	0.51	NS	0.006
L –Drum bone	1.23	1.24	1.26	1.26	1.28	1.30	NS	1.26	1.26	NS	0.008
Wings	7.57	7.48	7.53	7.54	7.65	7.63	NS	7.54	7.59	NS	0.019
Back and rack	16.74	16.31	16.57	16.52	16.28	16.53	NS	16.47	16.51	NS	0.053

[1]SEM = Standard error of the mean.

[a-c] Means in the same row (within dietary treatments) with common superscripts do not differ significantly ($P < 0.05$).

* = Linear regression with ($P < 0.05$).

** = Quadratic regression with ($P < 0.05$).

TABLE 8.9. Effect of pea inclusion and dietary amino acid levels on cause of mortality and culls (% of birds placed) from 0 to 35 d of age

Cause of mortality	Pea inclusion (g/kg)							Amino acid level			SEM[1]
	0	150	300	450	600	750	*P* value[2]	0.85	1.00	*P* value[2]	
Metabolic[3]	3.97	4.14	3.45	3.28	2.59	3.62	NS	3.91	3.10	NS	0.309
Skeletal[4]	0.52	0.86	0.69	0.34	0.34	0.52	NS	0.40	0.69	NS	0.139
Infection[5]	4.14	6.21	3.97	4.31	3.28	6.55	NS	4.83	4.66	NS	0.388
Unknown	1.21	0.52	1.38	1.38	0.52	0.69	NS	0.63	1.26	NS	0.156
Other[6]	0.34	1.38	0.52	0.52	0.34	0.69	NS	0.75	0.52	NS	0.123
Total	10.17	13.10	10.00	9.83	7.07	12.07	NS	10.52	10.23	NS	0.560

Values listed for means and standard SEM based on actual data.
[1] SEM: pooled standard error of the mean (N = 60).
[2] Values for the *P* based on log–transformed values.
[3] Metabolic diseases: ascites, sudden death syndrome.
[4] Skeletal: rotated tibia, spondylolithesis, tibial dyschondroplasia, valgus–varus.
[5] Infectious: arthritis, osteomyelitis, polyserositis, peritonitis.
[6] Other: accidental death, pendulous crop, twisted gastrointestinal tract.

9.0. THE EFFECT OF FEEDING FIELD PEA ON THE GROWTH AND METABOLISM OF BROILER BREEDER PULLETS

9.1. Abstract

A trial was conducted to determine the effect of feeding pea with slowly digested starch on the growth, blood glucose, plasma corticosterone, liver weight, and feed retention of broiler breeder pullets during the rearing period. A total of 192 day–old–female Ross 308 broiler breeder chicks were randomly assigned to 12 pens. Pea and wheat were used to formulate experimental diets containing slow digested starch (SDS) and rapid digested starch (RDS), respectively. Chicks were fed a starter pea–wheat–based diet for two wk (ad libitum) followed by either a pea or wheat based diet (6 pens per treatment). On the third wk, feed intake was restricted and chicks were fed every day (ED) for a wk as a transition to every–other–day (EOD) feeding from wk 4 until the end of the experiment at 12 wk of age. Body weight, determined weekly, and uniformity assessed at 3, 6, 9, and 12 wk of age were not affected by treatment and approximated the breed standard. Blood glucose was determined in14 regular intervals during a two–day feeding cycle at 6, 9, and 12 wk of age and the average blood glucose level was significantly lower in pea–fed pullets than wheat–fed pullets at 1, 2, 4, 6, 20 and 40 h after feeding. Plasma corticosterone determined at 10 intervals before and after feeding at 12 wk of age was not affected by treatment; at 1 h after feeding, pullets fed the pea diet had a numerically lower concentration than birds fed the wheat diet (P = 0.07). Feed retention in the crop and small intestine were not affected by treatment and the crop content was minimal by 24 h after feeding for both treatments. The relative–liver weight

at 12 wk of age was higher for wheat– than pea–fed pullets. In summary, this experiment demonstrates that field pea can be fed as a main feed ingredient for broiler breeder pullets and that feeding SDS from pea can alter the rate of digestion and postprandial metabolism.

Key words: broiler breeder, rapid or slow digested starch, performance, metabolism

9.2. Introduction

The genetic selection of broilers for fast and more efficient growth is associated with an increase in the severity of feed restriction required to maintain reproductive capacity in broiler breeders. If body weight is not appropriately controlled, abnormal ovarian development in broiler breeder hens results in an increased incidence of double follicular hierarchies and multiple ovulations, which ultimately reduces both the production of total and settable eggs. The other undesirable effects of overweight breeders are lameness and increased mortality (Katanbaf et al., 1989; Robinson et al., 1993; Chen et al., 2006). In order to avoid these performance and health problems, uniform target weights must be achieved using quantitative and qualitative feed restriction.

The target body weight at any particular age and uniformity of the flock are important criteria in broiler breeder production. Therefore, feed restriction is used to maintain 85% of the birds within flock body weight recommended for a specific age. Sexual maturity of hens is also regulated by feed restriction (Leeson and Summers, 2000). Feed restriction is most severe during the brooding and rearing period, but is

maintained through the breeding period as well. There are different methods of feed restriction that are applied to broiler breeders. Commonly an every–other–day regime (**EOD**) is used because it improves flock uniformity. In any feed restriction program, birds are fasting for a period of time. For instance, birds on EOD feed program are fed only once every 48 h and the amount of feed allocation during brooding and rearing could be less than 50% of their expected ad libitum feed intake (Savory et al., 1996; de Jong et al., 2002; Chen et al., 2006).

Feed restriction is associated with marked changes in bird metabolism during feeding and subsequent fasting periods (de Beer et al., 2008). These changes in metabolism are associated with hormonal changes that are based both on feeding status and diurnal patterns (Buyse et al., 2000; Kita et al., 2002). For instance, catabolism will be the main metabolic process during fasting, and lipogenesis will be switched to lipolysis (Buyse et al., 2000; Richards et al., 2003). Feed restriction and subsequent bird hunger can cause chronic stress in broiler breeders that is associated with an increase in plasma corticosterone (Nir et al., 1975; Mormède et al., 2007).

Despite the positive effects of feed restriction on health, production, and reproduction, there may also be a physiological stress associated with bird hunger (Mench, 2002; de Jong et al., 2003). One method of assessing stress in feed restricted broiler breeders is plasma corticosterone concentration. Feed restriction elevated plasma corticosterone concentrations, which may be an indication of chronic stress (Hocking et al., 1996; de Jong et al., 2002, 2005). However, it is not clear if the effects of feed restriction on plasma corticosterone reflect metabolism changes or physiological stress (de Jong et al., 2003).

Although, the effects of different feeding regimes on broiler breeder performance and welfare have received much attention (Robinson et al., 1992; Hudson et al., 2001; de Jong et al., 2003; Renema and Robinson, 2004; Tolkamp et al., 2005; Chen et al., 2006; de Beer et al., 2008, Ekmay et al., 2010), the effects of diet feedstuffs on breeder performance and metabolism are not well documented. Feedstuffs vary in total digestible nutrient content as well as the rate at which nutrients are digested in the small intestine. For instance, the rate of starch digestion varies among feedstuffs (Yutste et al., 1991; Weurding et al., 2001). These results were confirmed in our laboratory using an in vitro assay (Chapter 3.0 through 6.0). A good comparison of relative rates of starch digestion is wheat that is rapidly digested vs pea that is slowly digested.

In broiler chickens, incorporating slowly digested starch in a diet results in positive production effects with the mechanism of action suggested to be either pre– or post–absorptive in nature (Weurding et al., 2003a, b). In respect to pre–absorptive effects, glucose would be available for the distal part of the small intestine and therefore less amino acids would be oxidized for gut energy demand. In regard to post–absorptive effects, gradual glucose release and absorption would result in lower but longer lasting blood glucose and insulin peaks after a meal (Björck, 2006). Transport and utilization of absorbed amino acids are also stimulated by insulin. It can also be hypothesized that the difference in physiological response between slowly and rapidly degraded starch may be greater in restricted–fed animals like broiler breeders than in ad libitum fed birds.

The objective of this study was to evaluate the effect of feeding diets different in starch digestion rate on growth and physiological parameters of restricted fed breeder pullets. It was hypothesized that feeding slowly digested starch (pea–based diets) during

the rearing period would maintain broiler–breeder growth and health, while at the same time altering metabolism in a fashion to enhance bird performance and reduce hunger stress.

9.3. Materials and Methods

The current research was carried out in accordance with the Guide to the Care and Use of Experimental Animals, Canadian Council on Animal Care (1993) and was reviewed and approved by the Animal Care Committee of the University of Saskatchewan.

9.3.1. Birds and Housing

A flock of 192 Ross 308 pullets was reared in 12 floor pens from d zero until 12 wk of age with 16 chicks per pen. Chicks were obtained from a commercial hatchery (Lilydale Inc., Calgary, AB. Canada) and the Parent Stock Management Manual, Ross 308 (Aviagen, 2006) was used as a guide for growth rate and other management procedures. The pen size was 2.0 × 2.3 m and each pen was provided with a trough feeder that provided 14 cm of feeder space per bird and a drinker with 6 nipples (Lubing–4087). Pens were bedded with an equal amount of straw (approximately 10 cm thickness). During the experiment, room temperature was initially 34°C on d zero and then was gradually decreased to 22°C by d 28 and maintained at this level for the remainder of the trial. The lighting program was 23L:1D (1 d), 21L:3D (2 to 5 d) followed by 8L:16D for the remainder of the experiment. Light intensity was initially 20 lx for 0 to 5 d and then 5 – 10 lx.

9.3.2. Experimental Diets

Feed allocation, the ingredient composition and nutrient content of the experimental diets are shown in Tables 9.1 and 9.2, respectively. The experimental diets were formulated to meet or exceed the nutrient specifications of female parent stock (Aviagen, 2007a) and feed allocation was based on Parent Stock Performance Objectives (Aviagen, 2007b) in order to reach target body weights. Wheat and pea were chosen because they are different in their rate of starch digestion; wheat as a source of rapidly digested starch (RDS), and pea as a source of slowly digested starch (SDS). Both wheat and pea were used in the starter diet (0 to 2 wk) in order to adapt pullets to the treatment grower diets (3 to 12 wk) that were formulated to contain wheat or pea as the only source of starch. Wheat and pea seeds were ground using a full circle pulverator–hammer mill (Model 160–D, Jacobson Machine Works, Minneapolis, Minn. 55427, USA) fit with a 4.0–mm screen–hole size before being mixed with other ingredients in a Hobart mixer (Model L–800, Hobart Canada, Don Mills, ON. M3B 1B1). The pea cultivar used in this experiment was Eclipse and wheat was feed grade of unknown cultivar. Feed was provided in mash form throughout the experiment.

The treatment diets were formulated to be isocaloric and isonitrogenous, and the calculated total starch in both diets was at approximately 410 g/kg. Other than DL–Methionine, pea was the only source of the protein in the pea–based diet; wheat and soybean meal supplied amino acids in the wheat–based diet. All birds were fed ad libitum for the first 2 wk, feed–restricted on an every–day (**ED**) basis for wk 3, and then fed one h after lights came on using an **EOD** program until the end of experiment. Birds were provided with free access to water at all times.

9.3.3. Data Collection

9.3.3.1. Body Weight and Uniformity

Birds were weighed every week on a pen basis and compared with the breed standard in order to ensure that the target body weight was met. Individual body weights were obtained for all birds at 3, 6, 9, and 12 weeks of age in order to determine flock uniformity. Birds were individually weighed 1 h before feeding on the day of feeding and the coefficient of variance (**CV**) was calculated for body weight. Mortality was recorded on a daily basis.

9.3.3.2. Blood Glucose

Blood glucose was measured at 6, 9, and 12 wk of age over a two–day period. Blood collection occurred at 1 h before feeding (light on) and then 1, 2, and 4 h after feeding; collection continued every 4 h until the end of 48 h. Blood was collected from 5 pullets from each treatment at each interval time (1 bird/replicate/interval). Different birds were randomly chosen for each interval time. A OneTouch® UltraMini™ Meter[2] was used to measure blood glucose. A drop of blood from the brachial vein was put on the screen and the digits in *mmol/l* were recorded. Blood collection for each time interval took less than 10 min and a great effort was made to catch pullets quietly and quickly.

9.3.3.3. Plasma Corticosterone Concentration

Blood samples were collected from five pullets from each dietary treatment (1 bird/replicate/treatment). They were taken from the brachial vein at 10 different sampling intervals during a two–day period at the end of the experiment (12 wk). Blood collection occurred at 1 h before feeding (at light on) and then 1, 2, 8, and 16 h after feeding;

[2] Life Scan, Inc., Milpitas, CA. USA.

collection then continued every 4 h until the end of 40 h. To prevent disturbance of pullets during blood sampling, an effort was made to maintain as quiet an environment as possible. Blood was collected in EDTA vaccutainer tubes (3 ml) and stored on ice during collection. Pullets were selected randomly per pen. Samples were centrifuged (3,000 rpm) and the plasma separated, frozen and stored at –20°C until analysis of corticosterone. A Double Antibody Rat Corticosterone Kit (ICN Pharmaceuticals Inc., Orangeburg, NY) was used to determine corticosterone levels in duplicate in enzymatically pre–treated plasma (Sorenson et al., 1997).

9.3.3.4. Feed Retention and Liver Weight

At the end of the trial (12 wk of age), one pullet from each of five replicates per treatment was randomly selected, weighed, killed via cervical dislocation, and the crop and intestinal tract were removed. The sampling intervals were 1 h before feeding and 1, 2, and 4 h after feeding and then every 4 h up to 44 h. The contents of the crop, proximal jejunum, distal jejunum, proximal ileum, and distal ileum were collected and weighed as is. The digesta content from each section of the small intestine was gently squeezed out using a roller vial. Livers from killed hens were excised and weighed; the relative liver (**R–liver**) weight was calculated as a percentage of live body weight.

9.3.4. Statistical Analysis

Statistical analyses were conducted using SAS software (SAS Institute Inc., 2008). Before the analysis, data were checked for normality using the PROC Univariate test. Body weight and uniformity data at each age were analyzed as a one–way ANOVA using the Mixed Procedure. The experimental design was a Completely Randomized Design with 6 replicates per dietary treatment (16 birds per replicate). The effects of

source of starch (RDS and SDS) on blood glucose level for each collection interval (14 intervals during 48 h) were statistically analyzed as one–way ANOVA using the Mixed Procedure of SAS. The experimental design was a Completely Randomized Block Design with 3 blocks (3, 9, and 12 wk of age) and 5 replicates per block. Plasma corticosterone, gut contents, and R–liver weight data were analyzed in one–way of ANOVA using 5 replicates for each dietary treatment. Differences were considered significant if $P \leq 0.05$ unless otherwise stated.

9.4. Results

9.4.1. Body Weight and Uniformity

The average body weights based on pen weights are shown in Table 9.3. Pullets fed the wheat diet were heavier at 4, 5, and 6 wk of age and lighter at 9 and 10 wk of age than pullets fed the pea diet. No differences between the two dietary treatments were noted at other ages. Both dietary treatments were at or above the body weight target of the breed standard (Figure 9.1). The effects of feeding pea– or wheat–based diet on flock uniformity are presented on Table 4. At all studied ages, there were no differences between pea and wheat treatments. Bird uniformity expressed as CV% ranged between 10.7 and 13.4% for both dietary treatments.

9.4.2. Blood Glucose

The effects of blocks (age) on the level of blood glucose were not significant; therefore blocks were removed from the statistical model. The average of blood glucose levels measured 14 times during a two–day period at 6, 9, and 12 wk of age is shown in Figure 9.2. Across ages, blood glucose concentration at 1 h prior to feeding was low in all birds (12.4 *mmol/l*). Immediately after feeding, blood glucose levels in pea– and wheat–

fed birds increased by 47 and 82%, respectively, compared with pre–feeding levels. Blood glucose levels in wheat–fed pullets were higher than pea–fed pullets at 1, 2, 4, and 8, 12, 20 and 40 h after feeding. As indicated, the extent of the blood glucose increase immediately after feeding was lower for the pea–fed birds, but the overall pattern for the two dietary treatments was similar. After the initial rapid rise, blood glucose levels decreased during the day of feeding to low levels during the initial night period. Blood glucose increased during the off feed day and the decreased again for the second dark period.

9.4.3. Plasma Corticosterone Concentration

Dietary treatment did not affect corticosterone levels over the two–day period associated with a single feeding at 12 wk of age (Figure 9.3). Numerically (P = 0.07), corticosterone rose immediately after feeding to a peak at 1h in wheat treatment whereas it declined in the pea treatment. Corticosterone levels for birds in both treatments fell to a minimum at 8 h after feeding. On the no–feeding day, corticosterone level increased to a level higher than seen on the feeding day for both treatments; numerically wheat–fed birds had a higher level compared with birds fed the pea diet.

9.4.4. Feed Retention in Crop and Small Intestine

There were no significant differences between pea– and wheat–fed birds for the wet content weight of small intestine sections regardless of time after feeding (data not shown). Crop content was only significantly different at 1 h after feeding with pea–fed pullets having more digesta than wheat–fed pullets (Table 9.6). In both dietary treatments, there was a progressive reduction in crop content (Figure 9.4). Crop content

in pullets fed pea and wheat reached minimum values by 24 h after feeding and small intestine segments reached minimum values by 28 and 32 h after feeding.

9.4.5. Relative Liver Weight

The relative liver weights for pea– and wheat–fed pullets at intervals during the 44–h collection period are presented in Figure 9.5. The R–liver weight increased from feeding to a peak 16 h later and then gradually declined during the remainder of the data collection period. In comparison to pre–feeding, the R–liver weight of wheat–fed pullets increased by 70% at 16 h after feeding. In contrast, the R–liver weight of pea–fed pullets increased by 35% at 16 h compared with the before feeding value. The proportional liver weights of wheat–fed pullets were heavier than livers from pea–fed pullets at all collection points except at 36 h after feeding. R–liver weights prior to feeding were similar for both treatments.

9.5. Discussion

This experiment examined the effect of two different sources of starch, slowly digested from pea and rapidly digested from wheat, on body weight, body weight uniformity, blood glucose level, plasma corticosterone concentration, feed retention in the digestive tract, and R–liver weight. The course of a single feeding cycle based on EOD program was applied and it is known that pullets fed EOD have recurring phases of both anabolism and catabolism between feeding periods (Buyse et al., 2000). The shift from anabolism to catabolism occurs when nutrients are no longer available from the digestive tract at sufficient levels to cover the bird's metabolic needs. The physiological responses to EOD feeding, such as blood glucose, plasma corticosterone concentration,

and liver weight, are ultimately influenced by the nutrient supply from the small intestine. Therefore, the rate of starch digestion and glucose absorption may alter these responses.

9.5.1. Body Weight and Uniformity

The results of this experiment show that including pea (85.3%) in broiler breeder diets had no adverse effect on target body weight during the rearing period (3 to 12 wk of age) compared to the breed standard. Even though, wheat–fed pullets had higher body weights in wk 4, 5, and 6, pea–fed pullets compensated by 7 wk of age and even become heavier at 9 and 10 wk of age. Overall, pullets fed pea– or wheat–based diets reached their target body weight with only minor differences between treatments. To the best of our knowledge, the only other study to have investigated the effect of feeding pea on broiler breeder performance was conducted by Kill and Savage (1992). They found that feeding 85, 60, and 66% of pea to broiler breeders from 0 to 8, 9 to 27, and 28 to 46 wk of age, respectively, had no adverse effects on body weight gain, egg production, egg weight, fertility, and hatchability. This experiment was the first to use pea as the only source of starch and protein in the grower diet (0 to 8 wk of age) of broiler breeders (dwarf ISA Vedette). The results of our study are in agreement with the finding of Kill and Savage (1992). However, it should be borne in mind that the breed of pullets and variety of pea used were different between these studies.

The uniformity of body weight for broiler breeder can be calculated as the percentage of birds that are within ±15% of the average flock body weight or as a coefficient of variation (Aviagen, 2007b). Flock uniformity is associated with bird productivity (e.g. delayed sexual maturity and lower peak egg production) and ease of feeding management, and is therefore an important parameter in broiler breeder pullets.

In the current study, the uniformity of pullets was not affected by dietary treatment at any age. It can be concluded that the nutritional value derived from the high dietary level of pea is applicable for pullets during the brooding and growing period as there was no detrimental effect of dietary treatment on body weight or uniformity. It is emphasized that in this experiment, pea was included as main feed ingredient to supply energy and amino acids to broiler breeder pullets from 3–12 wk of age.

9.5.2. Blood Glucose

The blood glucose level in chickens is approximately 14 *mmol/l* and it is affected by feed intake and the nature of feed formulation (Hazelwood, 1986). Blood glucose levels in birds are higher than in mammals and can be quite variable. Levels of blood glucose as low as 6.0 *mmol/l* and high as 19.5 *mmol/l* have been documented (Hazelwood, 2000). The level of blood glucose is affected by feeding program, particularly if the bird is ad libitum or feed–restricted. Moreover, certain hormones such as insulin, glucagon, corticoids, and glucocorticoids also affect blood glucose level (Hazelwood, 1986).

In the present research, blood glucose level rose quickly after feeding for both treatments, but the degree of response was markedly lower for birds fed the pea than wheat diets. With the exception for the 16 h collection, blood glucose was higher for wheat–fed birds than pea–fed birds up to 20 h after feeding. The overall blood glucose response to feeding is similar to that found by Beer et al. (2008), who compared every day and every other day feeding. Blood glucose rose similarly for both pea and wheat treatments during the second day reflecting an expected circadian rhythm (Twiest and Smith, 1970). During the following night blood glucose declined, and at 40 h after

feeding, pea–fed pullet values were 7% higher than for wheat–fed birds. Because the digestive tract had long since been empty at this point, the source of glucose would have to be from de novo synthesis or glycogen. The difference in blood glucose at 40 h suggests that feeding pea results in more effective glucose utilization or glycogen storage than for wheat–fed birds. Even though insulin release in birds is less sensitive to blood glucose level than in mammals, the EOD program has a greater effect on insulin level than every day feeding (de Beer et al., 2008). This suggests that the lower post prandial glucose resulting from feeding pea would also alter the insulin response and bird metabolism. The reduced blood glucose response from feeding pea reflects the slower digestion rate of its starch in comparison with wheat (Weurding et al., 2001). To the best of our knowledge, no experiment has examined the effect of feeding starch with variable digestion rates on blood glucose level in broiler breeder chickens.

9.5.3. Plasma Corticosterone Concentration

Increased plasma corticosterone is known to be one of the physiological responses to hunger (Nir et al., 1975). This increase has been confirmed in broiler breeders where feed restriction has been associated with elevated corticosterone levels (Hocking et al., 1996; Mormède et al., 2007; de Beer et al., 2008). This suggests that one of the drawbacks of feed restriction is the associated stress, particularly during the brooding and rearing period, when feed restriction is most severe and birds are frequently fed EOD. Management practices that reduce the negative effects of feed restriction and maintain the target performance have been investigated. For example, diluting feed with indigestible fiber had no adverse effects on growth curve and uniformity in ad libitum fed chicks

during the rearing period (Tolkamp et al., 2005). However, other studies have reported no effect of diluted feed on plasma corticosterone (de Jong et al., 2005).

In the current research, it was hypothesized that feeding a slowly digested starch source would reduce the stress associated with EOD feeding of broiler breeder pullets and reduce corticosterone levels. However, this hypothesis could not be confirmed since feeding pea (slowly digested starch) only numerically (P = 0.07) reduced corticosterone at 1 h after feeding. It is of interest to note that on the non–feeding day, corticosterone levels for the pea fed pullets were numerically lower than for the birds fed the wheat based diet. A factor that may have prevented a statistically significant effect is the variable nature of corticosterone levels. Despite a considerable effort to minimize the stress of handling during blood collection, variability in blood corticosterone levels was high. The rapid nature of the corticosterone response and resulting variability have been reported previously and are reasons that blood corticosterone is not always an adequate measure of stress in birds. However, the numerical decrease in corticosterone levels for the pea fed birds at the above mentioned times suggests that stress may be decreased and that further research is warranted to confirm or reject this possibility.

9.5.4. Feed Retention in Crop and Small Intestine

The content of crop and small intestine were collected in 14 intervals as described earlier. There were no effects of experimental treatments on the content of crop, and proximal and distal sections of jejunum and ileum. Data indicate that there was a quick reduction in crop contents by approximately 8 h after feeding, which is in agreement with the finding of de Beer et al. (2008). It is also confirmed that the crop is emptied by 24 h after feeding in pullets fed EOD. Even though feed was consumed quickly on the feeding

day, the small intestine was provided with a continuous supply of feed for an extended period, as the crop was able to store and progressively release a large amount of feed.

9.5.5. Relative Liver (R–liver) Weight

In general, pullets fed EOD have recurring phases of both anabolism and catabolism between feeding periods (Buyse et al., 2000). Soon after the feeding period, excess nutrients are stored in the form of glycogen and lipid (primarily in the liver), whereas during fasting, these stored nutrients are mobilized. Soon after the feeding period glycogen is synthesized in the liver, however the capacity of the liver for glycogen storage is limited and extra dietary energy is converted to triglycerides. Increased liver glycogenesis and lipogenesis in turn results in increased liver weight. It has been documented that birds fed ad libitum have a smaller liver size compared with feed restricted birds because of less need to store energy until the next meal (Muiruri et al., 1975). Liver weight is also affected by feeding frequency in feed restricted broiler breeders. The R–liver weight in 16–wk–old pullets fed EOD is higher than pullets with ED feeding and the increase in liver weight is related to liver glycogen and lipid content (de Beer et al., 2007). This finding demonstrates that the frequency of nutrient supply is a main factor affecting liver size and fluctuations in liver size reflect fluctuations in nutrient supply. Our results agree with findings of de Beer et al. (2007) who found that the R–liver weight in breeder hens rises after feeding. The current study further demonstrates that smaller changes in R–liver occured in pullets fed a pea diet compared with those from birds fed a wheat diet. This finding supports an effect of SDS on bird metabolism compared to RDS. Whether there is a metabolic or other benefit due to a smaller increase in R–liver requires further investigation. Also in this research the content

of the small intestine reached minimal levels between 28 and 32 hours after feeding, but it is probable that the level of nutrient availability required for anabolism was reduced well before this time. The finding that liver weight decreased 16 hours after feeding is in agreement with this suggestion.

In summary, our research showed that field pea is a suitable feed ingredient for broiler breeder pullets and can be used as the main source of dietary energy and protein. Feeding pea resulted in markedly lower post–prandial blood glucose levels and reduced liver weight changes between feedings that can likely be attributed to the slowly digested nature of pea starch. Numerical differences in corticosterone concentration support the concept that feeding a slowly digested feed ingredient like pea may reduce stress in bird, but additional research is required to confirm this result. Future research should also examine indicators of satiety and behaviour to more definitively establish whether feeding pea has beneficial effects on bird welfare. Future studies should include the breeding period in order to evaluate the effect of including pea in broiler breeder diets.

TABLE 9.1. Feed allocation for broiler breeder pullets fed pea or wheat based diets

Age (wk)	Feed (g/bird)[1]	Feeding program
1	–	ad libitum
2	–	ad libitum
3	29	Every day
4	66	Every other day
5	78	Every other day
6	88	Every other day
7	92	Every other day
8	96	Every other day
9	100	Every other day
10	104	Every other day
11	108	Every other day
12	112	Every other day

[1] Based on feed recommendation for Ross 308 broiler breeders, Aviagen (2007).

TABLE 9.2. Ingredient composition and calculated nutrient content (%) of experimental diets

Feed ingredients	Starter diet (0 – 2 wk)	Grower diets (3 – 12 wk)	
		Wheat–based diet	Pea–based diet
Pea	50.64	–	89.25
Wheat	37.05	67.18	–
Soybean meal	5.74	18.00	–
Oat hulls	–	7.00	–
Canola oil	2.00	2.30	5.00
Dicalcium phosphate	1.69	1.56	1.55
Ground limestone	1.46	1.27	1.25
Sodium chloride	0.33	0.44	0.45
Vitamin–mineral premix[1]	0.50	0.50	0.50
Choline chloride 60%	0.10	0.10	0.10
DL–Methionine	0.27	–	0.25
L–Threonine	0.07	–	–
Enzyme[2]	0.05	0.05	0.05
Coccidiostat[3]	0.10	0.10	0.10
Acid insoluble ash	–	1.50	1.50
Calculated nutrient content			
ME (kcal/kg)	2800	2797	2752
Crude protein (N × 6.25)	19.85	19.57	19.72
Starch	47.42	41.99	42.75
Calcium	1.00	0.90	0.90
Available phosphorus	0.45	0.42	0.42
Crude fat	3.61	4.17	6.08
Crude fiber	4.05	4.80	4.80

[1]Vitamin–mineral premix provided the following per kilogram of complete diet: vitamin A, 11000 IU; vitamin D, 2200 IU; vitamin E, 30 IU; vitamin K_3, 2 mg; biotin, 0.15 mg; niacin, 60 mg; pyridoxine, 4 mg; riboflavin, 6.0 mg; thiamine, 1.5 mg; pantothenic acid, 10 mg; folic acid, 0.6 mg; vitamin B_{12}, 0.02 mg; copper, 10 mg; manganese, 80 mg; iron, 80 mg; zinc, 80 mg; iodine, 0.8 mg; and selenium, 0.3 mg.

[2] Avizyme 1302, Danisco Animal Nutrition, Marlborough, Wiltshire, UK.

[3] Coccistac, Phibro Animal Health, Ridgefield Park, NJ, USA.

TABLE 9.3. Body weight of broiler breeder pullets (g) fed pea (SDS) or wheat (RDS) based diet

Age (wk)	Pea–based diet	Wheat–based diet	*P*–value	SEM[3]
3[1]	362	371	NS	2.8
4[2]	429	449	0.002	3.6
5	543	562	0.002	3.6
6	647	660	0.035	3.4
7	795	796	NS	3.0
8	901	894	NS	3.3
9	1030	1012	0.035	4.5
10	1117	1093	0.030	5.7
11	1238	1218	NS	6.0
12	1356	1342	NS	5.1

[1] Data shown represent means of 96 individual birds per treatment at 3, 6, 9, and 12 wk of age.
[2] Data shown represent means of 6 replications per treatment with 16 birds each at 4, 5, 7, 8, 10, and 11wk of age.
[3] SEM–pooled standard error of the mean.

TABLE 9.4. Body weight (BW) means and relevant coefficients of variation (CV)[1] of broiler breeder pullets at 3, 6, 9, and 12 wk of age fed pea– (SDS) or wheat– (RDS) based diets

Age (wk)	Pea–based diet				Wheat–based diet				SEM for CV
	BW (g)	SEM[2]	*n*	CV (%)	BW (g)	SEM	*n*	CV (%)	
3	362	4.0	95	10.7	371	4.7	95	12.3	0.87
6	647	7.5	95	11.4	660	7.5	93	10.9	0.72
9	1030	13.1	95	12.4	1012	12.1	93	11.6	0.53
12	1356	18.7	95	13.4	1342	17.4	92	12.5	0.43

[1] CV was determined by individually weighing birds per treatment at each interval and the effects of treatments were not significant ($P \leq 0.05$).
[2] SEM–standard error of the mean.

TABLE 9.5. Effect of feeding pea– and wheat–based diet on the wet weight of crop contents (g) for broiler breeder pullets at 12 wk of age

Time[1] (h)	Pea–based diet	Wheat–based diet	*P*–value	SEM[2]
–1	2.0	0.3	NS	0.88
1	149.5	102.9	0.016	1063
2	141.9	158.4	NS	11.44
4	121.0	122.8	NS	11.03
8	113.8	120.5	NS	9.77
12	98.2	95.9	NS	7.69
16	48.3	61.0	NS	4.93
20	19.6	34.0	NS	4.11
24	5.3	5.4	NS	1.12
28	8.4	11.7	NS	3.30
32	28.8	16.4	NS	4.60
36	9.7	10.8	NS	3.26
40	1.2	2.1	NS	0.52
44	1.0	0.5	NS	0.42

[1] Time before and after feeding in hours.
[2] Pooled SEM (n = 10).

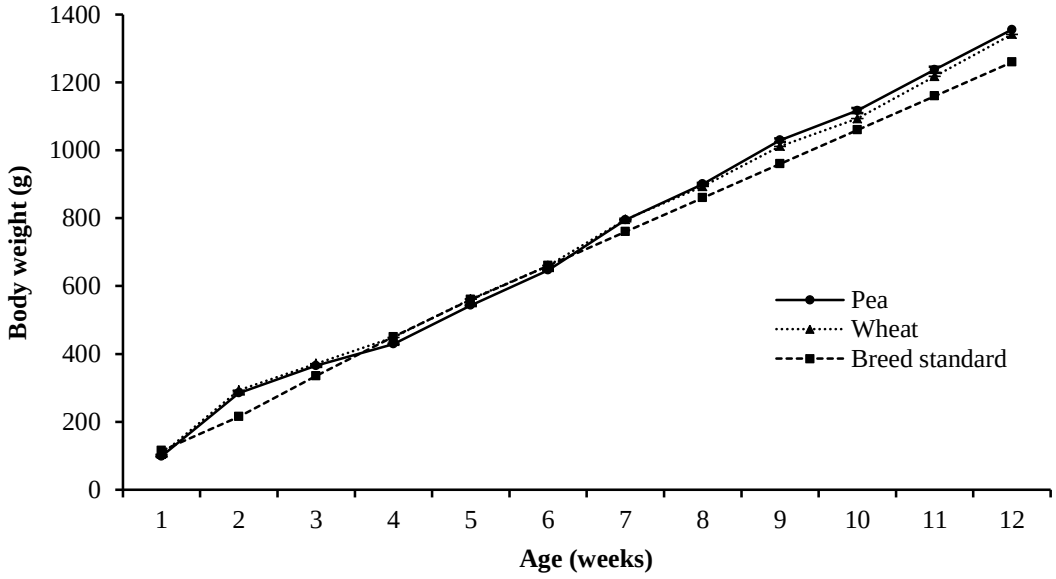

FIGURE 9.1. Mean body weight of pullets (g) fed every–other–day, restricted quantity of pea (SDS) or wheat (RDS) based diet compared to breed standard. Breed standard was based on Parent Stock Performance Objectives, Ross 308, Aviagen (2007).

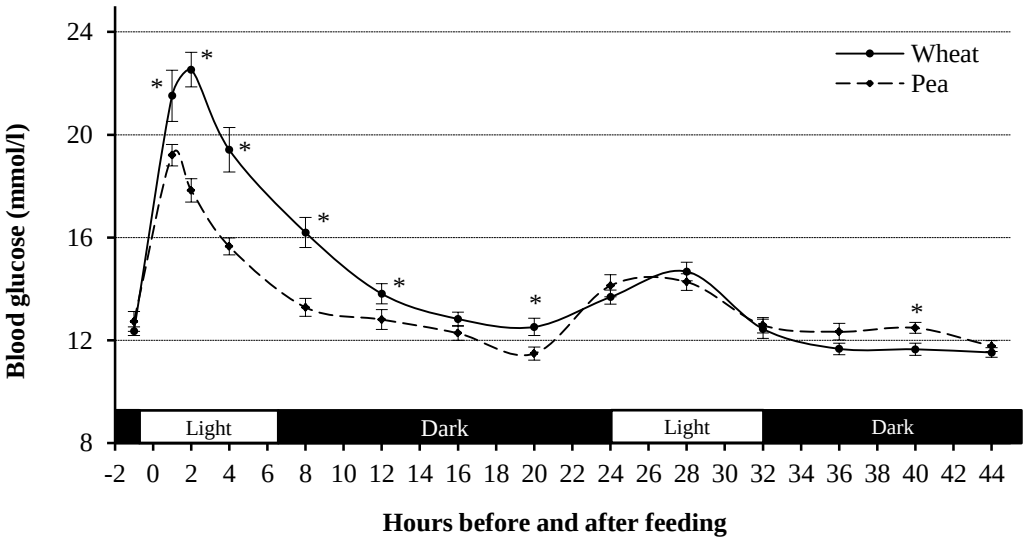

FIGURE 9.2. Blood glucose (*mmol/l*) in broiler breeder pullets fed pea (SDS) or wheat (RDS) as the only source of dietary starch based on measurements made at 6, 9, and 12 weeks of age. Pullets were fed every–other–day. Values are means of 15 observations for each treatment at each time point. Bars represent SEM; an asterisk (*) indicates time points at which a difference ($P \leq 0.05$) was found between pea and wheat treatments.

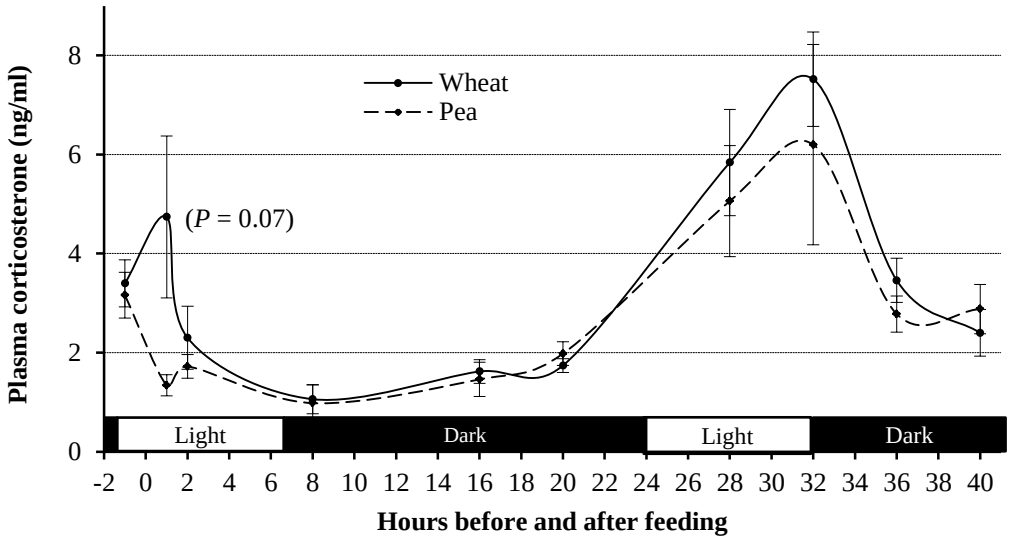

FIGURE 9.3. Average of plasma corticosterone (*ng/ml*) at 12 weeks of age in broiler breeder pullets fed pea (SDS) or wheat (RDS) as the only source of starch. Pullets were fed every–other–day. Data are means of 5 observations for each treatment at each interval point. Bars represent SEM.

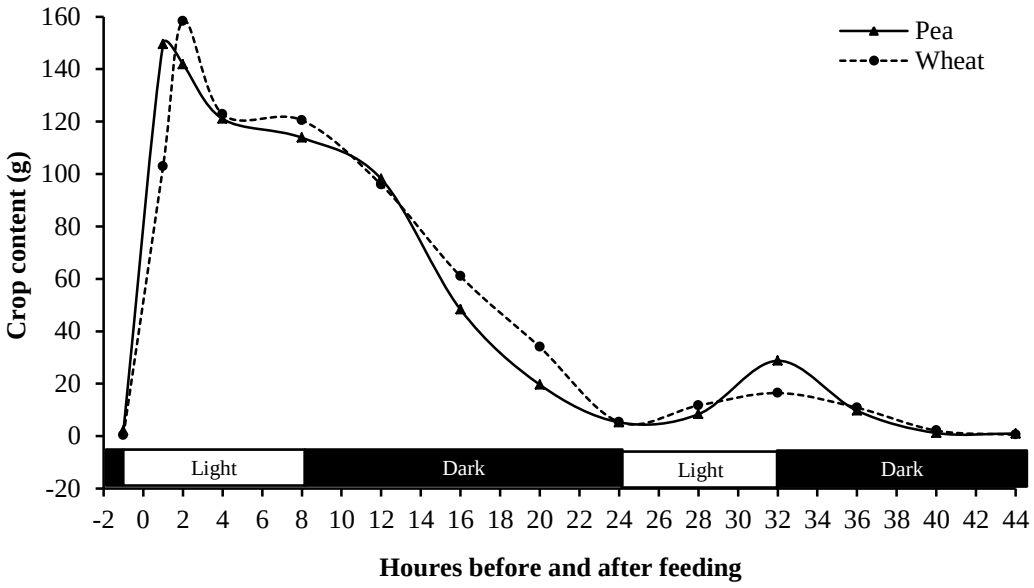

FIGURE 9.4. Feed content of crop after a meal of pea– or wheat– based diet. Pullets were fed every–other–day and feed allocation was (112 g/bird) at 12 weeks of age. Data are means of 6 observations for each treatment at each interval point.

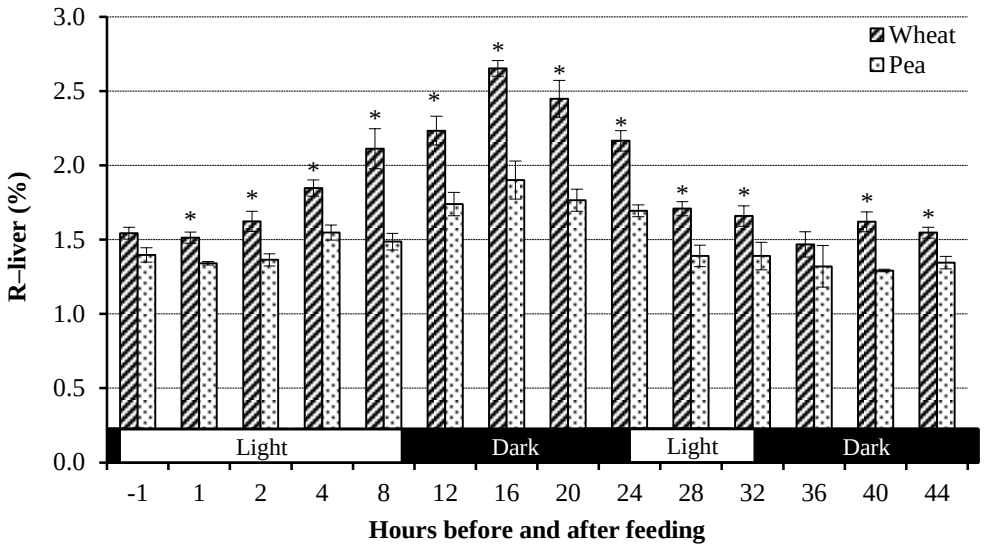

FIGURE 9.5. Relative liver (R–liver) weight (%) at 12 wk of age of broiler breeder pullets fed pea or wheat as the only source of starch. Hens were fed every–other–day. Data are means of 5 observations for each treatment at each time point. Bars represent SEM. An asterisk (*) indicates time points at which a significant ($P \leq 0.05$) difference was found between pea–fed and wheat–fed pullets.

10.0. OVERALL DISCUSSION

10.1. Introduction

The cost of animal production is mainly affected by the price of feed. Corn and soybean as the classical starch and protein sources for poultry, are warm season crops and not well suited to temperate regions of the world. For that reason, they are imported for poultry feed inclusion, at times at high prices. In some countries such as in Europe, pea has been widely included in animal feed. However, it has not been used extensively as an ingredient in the Canadian feed industry. Field pea (*Pisum sativum* L.) as a homegrown feedstuff can be included in poultry diets as a valuable ingredient supplying both dietary energy and protein (Gatel, 1994; Castell et al., 1996; Hickling, 2003). The inclusion of pea as a feed ingredient could substitute for other classical feed ingredients, particularly imported ingredients. The nutrient profile of pea is suitable for most poultry production, but it has not replaced soybean meal and corn due to incomplete and variable poultry nutrient data and limited industry experience including pea as a feed ingredient for poultry.

The nutritional evaluation of pea for poultry has been mostly investigated elsewhere, but under Canadian conditions foreign data are not sufficient for accurate feed formulation. Different pea cultivars and growing conditions may affect the nutrient composition and availability of pea for poultry. The potential nutritional value of Canadian pea for poultry has not been completely investigated. For the experiments reported in this thesis, there were three overall objectives. The aim of the first two experiments conducted was to study the effects of various feed processing on nutrient digestibility of pea (**Chapter 3 and 4**). The second goal investigated in **Chapters 5 and**

6 was to evaluate the interaction between Canadian grown pea cultivar and feed processing on pea nutrient digestibility. The final objective was to examine whether the response to amino acid levels in laying hens (**Chapter 7**) and broiler chickens (**Chapter 8**) is affected by the rate of starch digestion. The performance and metabolism of broiler breeder pullets as affected by feeding SDS from pea was reported in **Chapter 9**.

10.2. Nutritive Value of Pea as Affected by Feed Processing

Feedstuffs are initially and routinely evaluated based on their proximate analysis value. However, total nutrient contents do not reflect the nutrient availability for an animal. Therefore biological methods such as metabolizable energy system and nutrient digestibility have been applied historically. Nutrient availability has been used in most feed evaluation systems in which nutrient content in diet, digesta, and excreta (as determined by the proximate analysis) are used to determine the digestibility for nutrients. The difference between the nutrient content of consumed feed and resulting digesta or excreta is used to calculate ingredient digestible values. Limited digestibility data for some nutrients in feedstuffs has been published in NRC (1994). In poultry feed formulation, metabolizable energy values of feed ingredients are either estimated from published or analyzed contents of nitrogen free extract, crude protein, and crude fat and published digestibility values for these nutrients.

Starch supplies more than 50% of dietary energy requirement in poultry diets. However, it is neither included in the proximate analysis nor considered in feed formulation. In the modern poultry feed industry, grinding and pelleting have been applied widely. However, the effect of feed processing on pea nutrient availability has not been clearly delineated. Therefore the first objective in this project was to investigate

the impact of feed processing on pea nutrient availability. Two experiments were conducted for that purpose (**Chapter 3 and 4**). In the first experiment, the effects of hammer–mill screen–hole size (3.2–, 6.4–mm) and feed form (cold pellet, mash) were studied. In the second experiment the effects of the same screen–hole sizes and pre–pelleting conditioning temperature were investigated.

Determining AME_n, protein, and starch digestibility was complicated as it is affected by many factors related to the feedstuff itself as well as experimental conditions. For this work, all digestibility trials were conducted using the same management procedures and breed, sex and age of chicks; pea samples also came from the same source. Data reported in **Chapter 3** demonstrate that the nutritive value of pea is improved by processing. The effects of screen–hole size and pre–pelleting conditioning temperature on variables studied were independent and no interaction was found. It confirmed the result of the first experiment with screen–hole size and cold pelleting. Because all diets were pelleted, diet condition and/or the friction associates with the size of die may equalize the effect of the two different screen–hole sizes on starch digestibility.

The results confirm that the effects of hammer–mill screen–hole size and feed form (cold–pellet; mash) on pea nutrient digestibility are independent. Pea AME_n, apparent ileal protein digestibility, and the extent of starch digestion are improved by fine grinding. The effect of small screen–hole size can be attributed to disrupting the pea seed and associated components, thereby decreasing particle size, and enhancing accessibility of digestive enzymes to starch and protein. Improving nutrient digestibility is a result of increasing the surface area of starch granules and protein chains available to digestive

enzymes. On the other hand, cold–pelleting affected the rate and extent of starch digestion, but not the AME_n or apparent ileal protein digestibility. This result may be related to the cold–pelleting procedure, which does not involve conditioning or pelleting at temperatures regularly used in commercial pelleting of poultry diets. Pelleting is the preferred method of feed processing as the significance of feeding pelleted diets to broilers is well recognized (Behnke, 1996). However, there are some negative possibilities in regard to nutrient availability as they have been affected by pre–pelleting conditioning temperature. Therefore, a balance between positive and negative effects of pelleting should be considered.

The interactive effects of hammer–mill screen–hole size and pre–pelleting conditioning temperature on nutrient digestibility of pea–based diet for poultry has not been studied previously. To the best of our knowledge, the experiment reported in **Chapter 4** is the first studying the effect of different pre–pelleting conditioning temperature on nutritive value of locally grown pea. It was clearly reported in this project (**Chapter 4**) that high pelleting–conditioning temperature adversely affect pea nutrient digestibility. The present study recommended 70°C as the pre–pelleting conditioning temperature for pea–based diets. This may indicate that 70°C is close to the optimum temperature of pea. Higher pre–pelleting conditioning temperature may have reduced the digestibility of starch as a result of the formation of RS. However, a quadratic effect of pre–pelleting conditioning temperature on AME was not supported by starch digestibility. The underlying physical and chemical changes of starch digestibility as a result of pelleting were not delineated clearly; more research is needed to clarify this point. Regardless of screen–hole size, data reported in Chapter 4 showed that increasing

pre–pelleting conditioning temperature from 60 to 92°C decreased AIPD. The effect of high pre–pelleting conditioning temperature is most likely related to protein denaturation and increased Maillard reactions. However, pre–pelleting conditioning time and moisture content during pelleting should be also considered.

10.3. Nutritive Value of Pea as Affected by Pea Cultivar and Feed Processing

Previous researchers have demonstrated that pea cultivars vary in starch and protein content (Gatel, 1994; Wang and Daun, 2004; Hickling, 2003; Hood–Niefer et al., 2011). In poultry feed formulation, differences among pea cultivars are often not considered. Therefore, the nutrient requirement for best performance may not be achieved. AME is the first value considered in poultry feed formulation. Starch is the major source of dietary energy in poultry diets and its digestibility is correlated with AME (Wiseman, 2000). Starch digestibility can be either determined using an in vivo method or predicted using an in vitro method. The effect of pea cultivar and feed processing as well their interaction on AME, apparent protein digestibility, and the rate, site, and extent of starch digestion in broiler chickens were examined in **Chapters 5 and 6**.

Starch is the major component of pea and cereal grains and it is packed in granules, which are mainly made up of amylose and amylopectin molecules. Both polysaccharides are composed mainly of glucose molecules. In the gastrointestinal tract, the susceptibility of starch granules to enzymatic attack is determined by its physical and chemical nature. This includes the size and shape of granules, the ratio of amylose to amylopectin, the crystalline structure, and lipid and protein encapsulation.

The in vivo method cannot be easily used to study starch digestion in a large number of samples. It is time consuming, costly, and requires a sophisticated technique. Therefore an in vitro assay was developed based on a procedure described by Bedford and Classen (1993) and a modified version of the method introduced by Englyst et al. (1992). Our modified method permits comparison of the kinetics of starch digestion in a large number of samples, and therefore the effect of different pea cultivars was investigated. Moreover, the effect of different techniques of feed processing on the rate and extent of starch digestion was examined as well. Even though the in vitro method was developed to stimulate the conditions of the digestive tract in chicken, it can never mimic the exact process in the gastrointestinal tract of the chickens. For example, passage rate and viscosity of a diet cannot be simply simulated by in vitro method. On the other hand, the in vitro experiment is further quicker, simpler, standardized, and more cost effective, and has no animal welfare implications than using the in vivo method.

In this project, the effects of pea cultivar and sieve–hole size and their interaction on in vitro starch digestion rate and extent were investigated. A separate experiment was also conducted to compare the kinetics of starch digestion of pea with cereal grains, barley, corn, and wheat. The latter experiment confirmed that pea starch is more slowly and digested to a lesser extent than the starch from the cereal grains tested (Carré et al., 1991; Gatel, 1994; Igbasan et al., 1997). This difference can be explained by species differences in starch granule structure (e.g. amylose and amylopectin ratio, starch encapsulation).

Most of previous research on pea has used either one or a limited number of pea cultivars or samples. The data from **Chapter 6** demonstrated variability in apparent

metabolizable energy values among pea cultivars. The lower value of AME in some pea cultivars may have been the result of low starch and protein digestibility. The different chemical and physical structure of starch and NSPs concentration may have increased digesta viscosity, which may reduce nutrient digestibility (Choct, 1997). Significant differences in the AIPD were found between pea cultivars, but the effect of screen–hole size and feed form were vice versa. The variability in apparent protein digestibility found between pea cultivars may be due to the differences in the level of amino acids and the concentration of ANFs.

The reported AME of different pea batches is variable and values range between 2600 – 3200 kcal/kg (Carré et al., 1991; Perez–Maldonado et al., 1999). This variation is mostly related to differences in pea cultivars and also the technical conditions of the experiments. In this research, samples of nine pea cultivars were tested and differences in nutrient digestibility were confirmed. In Chapter 6, more than 130 kcal/kg (DM) difference in AME_n value among pea cultivars was reported. The difference between pea cultivars with different screen–hole size and feed form was more pronounced. However, the maximum AME difference among pea cultivars, screen–hole size, and feed form was 800 kcal/kg. This confirms the major effect of feed processing on pea nutritional value. The overall mean of AME of pea determined in this project was in agreement with current tabulated value 2,650 kcal/kg.

Some of the implications of feed processing on the nutritive digestibility of pea for poultry have been elucidated in the current project. The combined data from Chapter 3 through 6 indicate that the effect of feed processing on pea nutrient digestibility was as expected. The small screen–hole size of hammer mill is for the best nutrient utilization.

Pre–pelleting conditioning temperature was also shown to have an effect on pea AME. Approximately 70°C is the optimum conditioning temperature for pelleting poultry diets formulated with high levels of pea. Our results revealed that the rate and extent of starch digestion and AME value for pea were affected by pea cultivar. The results indicate that pea starch is slowly digested in the small intestine with up to 20% of the starch digested in the ileum. The slowly digested nature of pea starch was confirmed in both in vitro and in vivo experiments.

10.4. Nutritive Potential of Slow Digested Starch from Pea

Following the in vitro and in vivo digestion studies, three separate performance experiments with laying hens (**Chapter 7**), broilers (**Chapter 8**), and broiler breeder pullets (**Chapter 9**) were conducted. The purpose of conducting those experiments was to fulfill the third objective in this project. It was to study and understand how SDS from pea affects amino acid utilization by laying hens and broilers and the performance and metabolism of broiler breeder pullets.

The hypothesis of the third strategy was based on the foundation of human nutrition. It has been documented that the potential benefits of SDS on health are linked to glucose metabolism (Jenkins et al., 2002; Björck, 2006; Lehmann and Robin, 2007). It was hypothesized that the rate of starch digestion and glucose absorption would impact bird metabolism and ultimately bird performance. It has been reported that performance of broiler chickens is improved by feeding a diets containing a mixed of SDS and RDS compared with a diet containing only RDS (Weurding et al., 2003). The nature of starch digestion may elicit different metabolic responses in the bird, sparing amino acids, improving glucose utilization, and providing a longer lasting insulin response.

In the small intestine, the rate of starch digestion and the rate of digesta passage modulate the amount of absorbed glucose in the distal section. RDS is mainly digested in the proximal small intestine with glucose mostly absorbed in the proximal section and low levels of glucose remaining the distal section. However, amino acids as well as glucose are used in the gut wall as a source of energy. Therefore, if amino acids are oxidized to supply the energy requirement of the gut wall, less amino acids will be available for protein synthesis, which could ultimately affect animal performance. If starch digestion could be partially shifted to the distal small intestine by feeding SDS diets, more glucose would be available for energy for enterocytes and less amino acid would be oxidized. However, if the rate of starch digestion is too slow, a proportion of starch would pass undigested to the hind gut (Englyst et al., 1992). In the hindgut, this fraction of starch could be fermented by the microbial action producing fermentation products such as VFAs, CO_2, and methane. Although VFAs can be used as an energy source, fermentation is not as efficient as direct glucose utilization. Furthermore starch fermentation in the ceca and colon is thought to be negligible in chickens compared with other species (Pesti et al., 2005). Therefore, starch not digested by the distal ileum would likely have little nutritional value for poultry.

In the small intestine wall, a portion of glucose is locally utilized as a source of energy. However, most of glucose is not completely oxidized and is metabolized to lactate and alanine. It has been reported that more than 30% of absorbed glucose is converted to lactate (Riesenfeld et al., 1982). Lactate in the cell wall and muscles is then transported to the liver where it can be converted to glucose again. Glucose absorption in excess of need could be stored as glycogen or converted to fatty acids. In the case of a

rapidly digested starch, more glucose would be converted to lactate than in the case of a slowly digested starch and also glucose would be less available to the terminal regions of the small intestine. It can be speculated that direct glucose utilization by the small intestine would be more efficient than conversion to lactate and reconversion to glucose.

The blood glucose level is related to the rate of glucose absorption. Therefore, a spike rise of plasma insulin occurs when blood glucose is rapidly increased. The high level of insulin, which results in a series of metabolic changes, drops quickly to a normal level in the post–absorption phase as glucose absorption is reduced. Because insulin level is low in the post–absorption, blood glucose cannot be utilized to supply energy demand in muscles. Therefore muscle cells utilize free fatty acids as a source of energy, but glucose is secured as an energy supply for the brain. The efficiency of supplying glucose through these metabolic processes is lower than utilizing glucose directly. On the other hand, a slow release of glucose into blood stream will result in a moderate, prolonged insulin response. Therefore, more glucose will be directly utilized and less fat will be synthesized. Because insulin level is prolonged with feeding of SDS, more amino acids may be used in protein synthesis and gluconeogenesis will be minimized. Therefore a prolonged and continuous glucose supply from the basolateral membrane is needed.

Based on the above mechanisms, the hypothesis that feeding SDS from pea would improve amino acid utilization was investigated for the laying hen (**Chapter 7**). Three levels of amino acid intake were chosen and were based on the ideal protein concept. It was anticipated that the chosen levels of amino acids would affect laying hen performance. In turn, it was hypothesized that the amino acid sparing effect of feeding SDS would result in a greater effect of feeding pea at low levels of dietary amino acids.

However, no interactions were found between dietary treatments and therefore the hypothesis was rejected. As the level of pea inclusion increased, hen body weight gain and egg weight also increased. It can be speculated that pea affected energy and protein utilization, possibly as a result of altered glucose metabolism. In general, this experiment showed that feeding diets containing up to 300 g/kg of pea had no detrimental effects on laying hen performance and may provide nutritional value beyond what is captured in existing pea nutrient profiles. These performance results are consistent with most previous research (Davidson, 1980; Castanon and Perez–Lanzac, 1990; Ivusic et al., 1994; Igbasan and Gunter, 1997a, b; Perez–Maldonado et al., 1999; Fru–Nji et al., 2007).

The hypothesis that SDS from pea may spare dietary amino acids was also tested in a broiler model (**Chapter 8**). Six levels of pea inclusion in diets with either 100 or 85% of recommended dietary amino acids (Aviagen 2007) were tested. Having a wide range of pea inclusion permitted a prediction for the maximum level of pea inclusion to be used in starter, grower, and finisher diets. The results of this experiment indicated that broilers are sensitive to the rate of starch digestion. Therefore, it can be speculated that as the level of SDS increased, a improved synchronization between glucose and amino acid absorption resulted in better performance. But at the higher level of pea inclusion, the synchronization may have been impaired again and some of starch fermented instead. Differences between the results of this experiment (**Chapter 8**) and Weurding et al. (2003) may be explained by the different ages and breed of birds, phase feeding, and feed formulation. Performance of broiler was depressed at high level of pea inclusion in the current study and the reason for this effect cannot be established based on the data collected. It is possible that at high levels of pea addition, amino acids beyond methionine

and threonine, which can be added in crystalline form, become limiting. Calculated amino acid levels support this suggestion.

Both experiments, laying hens (**Chapter 7**) and broilers (**Chapter 8**), reported in this thesis failed to demonstrate that feeding SDS from pea spares amino acids. However there were some indications that pea provided nutritional benefit beyond those used in feed formulation. We could not determine whether this was associated with SDS or not. It can be speculated that energy efficiency may be improved as a result of dietary energy and protein synchronization. Regardless our results showed that, pea can be included at relatively high levels in poultry feeds without loss of growth or reproductive performance.

Modern broiler breeders are severely feed restricted and therefore the metabolic consequences of chronic fasting can be elucidated. After a meal, the amount of absorbed glucose that is not utilized by the small intestinal is released from the gut wall to the blood. Therefore, blood glucose levels rise and insulin release occurs in response (Björck, 2006). The transportation and uptake of glucose and amino acids by body cells are regulated by insulin; hence the level of these nutrients in the bloodstream are lowered. In this situation, the required energy for metabolism would be mainly supplied by glucose.

Glucose is the main energy source for the animal body. Glucose is mainly utilized by body cells to supply the energy necessary for processes in the body. The extra glucose that is not utilized in the body metabolism is stored in the liver and muscles in the form of glycogen. However, the liver and muscles have a limited capacity to store glucose as glycogen and therefore the remaining glucose is transformed to fatty acids and stored in the form of fat. Muscles are the main place for glucose and amino acids uptake. Amino

acids are used for protein synthesis. During the post absorptive period, stored energy in the form of glycogen and fat will be catabolized to supply energy requirements. In this situation liver and muscle glycogen can be catabolized to raise the level of blood glucose level. As mentioned above, the capacity of liver to store glycogen is limited. Therefore, increasing glucagon level while insulin level is low would stimulate gluconeogenesis from muscle amino acids and production of fatty acids and glycerol from adipose tissues.

An experiment was performed to study the effect of SDS from pea on broiler breeder pullets during the period of severe feed restriction during the rearing period (**Chapter 9**). Body weight and uniformity for pullets fed pea–based diet (893 g/kg) were similar to those fed a wheat–soybean based diet. The rate of starch digestion affects the rate of glucose absorption. It was shown that pea starch reduced the post–prandial glucose peak and prolonged circulating blood glucose levels. For the purpose of the current studies, blood glucose level as a measure of the bird metabolism provided useful information on the effect of feeding SDS from pea on bird metabolism. In conclusion, feeding pea at high levels had an important impact on the postprandial nutrient metabolism of meal–fed broiler breeder pullets. It can be suggested that feeding pea to breeders may benefit bird satiety and welfare. The body weight and uniformity of pullets during rearing period from 0 to 12 wk of age provided some evidence for the use of pea in breeder diets. Based on reduced variation in blood glucose level and lower relative liver weight (R–liver), it can be speculated that pea–fed pullets would be less stressed (physiologically) than wheat–fed pullets.

10.5. Conclusions

This thesis studied the effects of pea cultivar and feed processing on the nutritional value of pea for poultry. The moderate metabolizable energy value and digestible protein of pea was confirmed. Feed processing, hammer–mill screen–hole size, feed form, and pelleting–conditioning temperature affected pea–feeding value. Overall, both particle size reduction and pelleting, regardless of pelleting temperature (cold vs. steam) increased AME_n, and protein and starch digestibility of pea for poultry. To the best of our knowledge, this work is the first to characterize the response to prepelleting–conditioning temperature on the feeding value of pea for poultry. The feeding value of pea for poultry was negatively affected by high pelleting–conditioning temperature. Pea starch degradation, rate and extent, were predicted using an in vitro procedure. Pea cultivar as well sieve–hole size affected the rate and extent of starch digestibility as studied in both the in vitro or in vivo models. Regardless of processing or cultivar, the nature of slow digested starch of pea compared with other grains, corn, barley, and wheat was observed. Considering the differences between pea cultivar and feed processing has the potential to alter the nutritional value of pea for poultry. The current research failed to establish the amino acid sparing benefits of feeding SDS from. However, it demonstrated that Western Canadian pea has nutritional value for different classes of poultry. Pea can be used at relatively high levels in poultry feeds without loss of performance. Formulating broiler diets with pea is age related as less inclusion for younger chicks is recommended. Diets for broiler breeder pullets can be formulated with pea as the main source of energy and protein supplemented with DL–Met with no negative effect on their

growth. Feeding pea at high levels had an important impact on the post-prandial nutrient metabolism of restricted–fed broiler breeder pullets.

10.6. Implications

Field pea production in western Canada has increased over the past two decades as farmers have realized the agronomic (crop rotation) and economic benefits of growing pea. At the same time, the cost and demand for feedstuffs has increased and pea offers has potential to be more extensively used in poultry feeding. In addition, benefits of feeding SDS on animal metabolism and performance have been suggested. These are the main factors that stimulated this project.

Feed processing has significant effects on pea nutritional value. Small screen–hole size is beneficial in terms of feeding value. Pea based diets should be pelleted with a pre–pelleting conditioning temperature around 70°C. High pre–pelleting conditioning temperature must be avoided. The poultry and feed industries should be aware of differences among pea cultivars. Cultivar and method of feed processing should be considered when poultry diets are formulated with pea. Laying hen producers can include up to 300 g/kg of pea in diets. Broiler producers should consider flock age when considering pea inclusion level in diets. For starter diets (0 to 10 d) a maximum of 300 g/kg pea inclusion is recommended, whereas up to 600 g/kg of pea can be used to formulate grower and finisher diets. DL–methionine, L–threonine, and L–tryptophan should be supplemented as needed. Producers for broiler breeder pullets can formulate diets with no limit for pea inclusion.

10.7. Future Research

In this project, two hammer–mill screen–hole sizes (3.2–, 6.4–mm) were evaluated in all conducted digestibility trials. It would be of interest to examine other practical screen–hole sizes as well as roller milling. New cultivars of pea are being bred and it is advised that they be routinely evaluated in poultry diets. The in vitro procedure that has been developed in this project was repeatable, simple, and less expensive. It can be used to screen a large number of samples or cultivars in a short period of time. However, it would be of value to establish a relationship between the in vitro and the in vivo starch digestion. Other techniques of feed processing should be further investigated. These include dehulling, micronizing, and extrusion. Another area that should be considered is using NSP–degrading enzymes in pea–formulated diets. Only apparent protein digestibility was studied in this research. Amino acid digestibility of pea cultivar should be investigated as well.

In this work, diets fed in performance trials were formulated using wheat, soybean meal, and pea as the main feedstuffs. Other feedstuffs that are available should be used to formulate poultry diets with pea. Therefore the interaction between other feedstuffs and pea could be examined. In this project the 300 g/kg of pea inclusion in laying hen diets was investigated using a phase feeding program. It would be interesting to know the maximum level of pea inclusion in laying hen diets. Determining the upper limit of pea inclusion for broilers in separate phase experiments based on the growing phase is also of interest in order to eliminate the effect of the previous period's diet. Feeding pea to broiler breeders may benefit bird satiety and welfare; further investigations are needed to understand the potential of feeding SDS from pea. These include measuring other

metabolites and hormones, abdominal fat, as well as bird behavior. Studying the effects of pea inclusion on broiler breeders in a full production cycle is needed.

11.0. REFERENCES

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15.0. APPENDIX

15.1. Supplemental Tables for Chapter 7.0

TABLE 15.1 Effect of strain, pea inclusion, and lysine level on daily feed intake of laying hens (g/hen/day)

Age (wk)	Strain		Pea inclusion (g/kg)			Lysine intake (mg/h/d)			SEM[3]
	A[1]	B[2]	0	150	300	700	780	860	
21–24	86.7[b]	99.0[a]	91.3	92.7	94.5	92.4	92.9	93.1	0.74
25–28	95.4[b]	107.6[a]	101.2	101.1	102.2	101.9	101.3	101.4	0.70
29–32	97.0[b]	111.0[a]	103.7	103.6	104.8	104.7	103.7	103.7	0.80
33–36	101.6[b]	116.3[a]	108.5	108.7	109.8	109.5	108.2	109.2	0.84
37–40	100.2[b]	112.9[a]	106.6	106.2	106.8	107.3	106.2	106.2	0.74
41–44	102.0[b]	112.6[a]	107.3	106.9	107.7	107.7	107.1	107.2	0.63
45–48	103.0[b]	113.7[a]	107.9	108.2	109.0	108.9	107.9	108.2	0.67
49–52	105.2[b]	115.8[a]	110.4	110.0	111.1	111.3	109.6	110.6	0.68
53–56	104.3[b]	113.1[a]	108.2	108.7	109.1	109.2	108.5	108.4	0.59
21–56	99.5[b]	111.3[a]	105.0	105.1	106.2	105.9	105.1	105.3	0.68

[a–c] Means within the same row and main effects with different superscripts differ significantly ($P \leq 0.05$).
[1] Hy–Line CV–20.
[2] Lohmann LSL–Lite.
[3] SEM–Pooled standard error of the mean (N = 90).

TABLE 15.2. Effect of strain, pea inclusion, and lysine level on the average body weight (BW) and body weight gain (BWG) of laying hens (kg)

Age (wk)	Strain			Pea inclusion (g/kg)				Lysine intake (mg/h/d)				SEM[3]
	A[1]	B[2]	*P* value	0	150	300	*P* value	700	780	860	*P* value	
20	1.30[b]	1.47[a]	< 0.01	1.38	1.39	1.39	NS	1.39	1.39	1.38	NS	0.009
44	1.63[b]	1.69[a]	< 0.01	1.65[b]	1.66[ab]	1.68[a]	0.01*	1.62[b]	1.67[a]	1.69[a]	< 0.01*	0.007
54	1.71	1.71	NS	1.68[b]	1.71[a]	1.73[a]	< 0.01*	1.66[c]	1.72[b]	1.75[a]	< 0.01*	0.008
20–54	1.55[b]	1.62[a]	< 0.01	1.57[b]	1.59[ab]	1.60[a]	< 0.01*	1.56[b]	1.59[a]	1.61[a]	< 0.01*	0.006
BWG	0.409[a]	0.238[b]	< 0.01	0.297[b]	0.326[a]	0.348[a]	< 0.01	0.269[c]	0.330[b]	0.371[a]	< 0.01*	0.012

[a–c] Means within the same row and main effects with different superscripts differ significantly ($P \leq 0.05$).
[1] Hy–Line CV–20.
[2] Lohmann LSL–Lite.
[3] SEM–Pooled standard error of the mean (N = 90).
*= Linear regression with $P \leq 0.05$.
BWG = Interaction Pea X Lys ($P = 0.0472$).

TABLE 15.3. Effect of strain, pea inclusion, and lysine level on THHP[1] (%) of laying hens

Age (wk)	Strain		Pea inclusion (g/kg)			Lysine intake (mg/h/d)			SEM[4]
	A[2]	B[3]	0	150	300	700	780	860	
21	82.9[b]	89.2[a]	85.9	84.9	87.2	85.0	86.8	86.1	0.73
22	94.3	95.4	95.3	94.6	94.7	94.0	95.3	95.3	0.39
23	97.5	97.3	97.2	97.2	97.8	97.2	97.6	97.3	0.26
24	97.8	97.0	97.4	97.3	97.5	97.7	97.6	96.9	0.26
25	97.0	97.4	97.1	97.2	97.3	97.2	97.5	96.9	0.25
26	95.7[b]	97.4[a]	96.8	96.1	96.8	96.8	95.9	97.0	0.28
27	95.0[b]	96.6[a]	95.7	96.0	95.7	95.4	95.7	96.2	0.33
28	95.6	96.8	96.1	96.1	96.4	95.9	96.4	96.2	0.34
29	95.1[b]	96.8[a]	95.8	96.0	96.0	95.5	95.7	96.6	0.34
30	94.7	96.0	95.6	95.3	95.2	95.5	95.1	95.5	0.36
31	94.3[b]	96.3[a]	95.7	95.2	94.9	94.4	95.7	95.8	0.41
32	94.2	96.0	95.6	95.0	94.6	94.3	95.7	95.2	0.41
33	92.6[b]	96.0[a]	95.1	94.1	93.6	94.0	93.9	94.9	0.40
34	90.8[b]	95.4[a]	93.5	93.0	92.7	93.0	92.9	93.3	0.46
35	91.2[b]	95.0[a]	92.8	93.1	93.3	91.9	93.4	93.9	0.43
36	90.8[b]	94.9[a]	93.2	92.0	93.2	92.1	92.3	94.1	0.48
37	89.5[b]	94.8[a]	92.2	92.2	92.1	91.6	92.0	92.9	0.47
38	89.3[b]	94.0[a]	92.7	91.2	91.0	91.4	91.5	92.0	0.49
39	88.4[b]	93.7[a]	92.4	90.8	89.9	90.6	91.4	91.1	0.53
40	88.0[b]	93.0[a]	90.7	90.0	90.7	89.7	90.6	91.1	0.51
41	86.4[b]	93.1[a]	90.9	89.2	88.9	89.7	89.2	90.3	0.55
42	86.6[b]	92.0[a]	90.2	87.8	89.9	89.3	87.9	90.7	0.57
43	86.5[b]	91.6[a]	88.8	88.5	89.8	88.6	88.8	89.7	0.56
44	85.8[b]	91.5[a]	89.3	88.2	88.5	87.7	89.1	89.1	0.55
45	85.2[b]	91.2[a]	87.8	87.9	88.9	87.7	88.2	88.6	0.54
46	83.5[b]	89.6[a]	85.7	86.4	87.5	86.0	85.8	87.9	0.58
47	84.6[b]	90.2[a]	86.8	87.5	87.8	86.8	86.7	88.7	0.58
48	83.9[b]	89.6[a]	86.2	87.2	86.7	85.7	86.5	87.9	0.55
49	82.6[b]	88.2[a]	85.2	85.3	85.5	85.1	84.9	86.1	0.52
50	83.0[b]	88.2[a]	86.1	85.9	84.7	85.5	84.8	86.4	0.62
51	82.5[b]	87.9[a]	85.1	85.3	85.2	84.0	85.3	86.3	0.54
52	83.3[b]	89.1[a]	86.9	86.6	85.0	86.1	85.6	86.8	0.53
53	82.5[b]	87.8[a]	84.6	84.7	86.3	84.1	85.2	86.2	0.52
54	80.8[b]	86.5[a]	84.0	83.5	83.4	84.0	83.1	83.9	0.58
55	81.7[b]	86.2[a]	83.5	83.1	85.1	83.2	83.3	85.1	0.56
Average	88.1[b]	92.4[a]	90.4	89.9	90.3	89.8	90.1	90.8	0.33

[a–c] Means within the same row and main effects with different superscripts differ significantly ($P \leq 0.05$).
[1] Total hen housed egg production.
[2] Hy–Line CV–20.
[3] Lohmann LSL–Lite.
[4] SEM–Pooled standard error of the mean (N = 90).

TABLE 15.4. Effect of strain, pea inclusion, and lysine level on THDP[1] (%) of laying hens

Age (wk)	Strain		Pea inclusion (g/kg)			Lysine intake (mg/h/d)			SEM[4]
	A[2]	B[3]	0	150	300	700	780	860	
21	82.9[b]	89.2[a]	85.9	84.9	87.2	85.0	86.8	86.1	0.73
22	94.3	95.4	95.3	94.6	94.7	94.0	95.3	95.3	0.39
23	97.5	97.3	97.2	97.2	97.8	97.2	97.6	97.3	0.26
24	97.8	97.0	97.4	97.3	97.5	97.7	97.6	96.9	0.26
25	97.0	97.4	97.1	97.2	97.3	97.2	97.5	96.9	0.25
26	95.8[b]	97.5[a]	96.9	96.3	96.8	97.0	96.0	97.0	0.27
27	95.1[b]	96.9[a]	96.0	96.3	95.7	95.8	96.0	96.3	0.33
28	95.8[b]	97.3[a]	96.5	96.5	96.6	96.5	96.6	96.5	0.30
29	95.2[b]	97.4[a]	96.2	96.4	96.2	96.1	95.9	96.8	0.30
30	94.9[b]	96.6[a]	96.0	95.7	95.4	96.1	95.3	95.8	0.34
31	94.4[b]	97.0[a]	96.1	95.7	95.3	95.2	95.9	96.0	0.37
32	94.3[b]	96.7[a]	96.0	95.5	95.0	95.2	96.0	95.4	0.39
33	92.7[b]	96.9[a]	95.6	94.6	94.1	95.0	94.2	95.2	0.38
34	90.9[b]	96.4[a]	94.1	93.7	93.1	94.1	93.4	93.5	0.44
35	91.3[b]	96.0[a]	93.5	93.7	93.7	92.9	93.8	94.2	0.43
36	90.9[b]	95.9[a]	93.8	92.7	93.7	93.2	92.7	94.3	0.44
37	89.7[b]	95.8[a]	92.8	92.8	92.5	92.6	92.5	93.1	0.47
38	89.5[b]	95.0[a]	93.4	91.8	91.4	92.4	91.9	92.3	0.48
39	88.5[b]	95.0[a]	93.1	91.7	90.3	91.7	91.9	91.6	0.52
40	88.2[b]	94.4[a]	91.5	91.1	91.1	90.8	91.2	91.8	0.51
41	86.5[b]	94.7[a]	91.8	90.3	89.5	90.7	89.8	91.1	0.57
42	86.8[b]	93.6[a]	91.0	88.8	90.5	90.4	88.5	91.6	0.57
43	86.8[b]	93.0[a]	89.7	89.8	90.2	89.6	89.5	90.6	0.55
44	86.0[b]	93.4[a]	90.3	89.4	89.1	88.9	89.9	90.0	0.56
45	85.5[b]	93.0[a]	88.8	89.1	89.7	88.8	89.2	89.5	0.59
46	83.9[b]	91.6[a]	87.1	87.7	88.4	87.6	86.8	88.7	0.59
47	85.0[b]	92.2[a]	88.2	88.8	88.7	88.5	87.7	89.6	0.60
48	84.2[b]	91.7[a]	87.6	88.4	87.8	87.5	87.6	88.7	0.59
49	82.9[b]	90.3[a]	86.4	86.6	86.6	86.9	86.9	86.9	0.57
50	83.4[b]	90.3[a]	87.5	87.1	85.8	87.3	85.8	87.3	0.62
51	82.9[b]	90.0[a]	86.5	86.5	86.3	85.8	85.8	87.2	0.58
52	83.7[b]	91.2[a]	88.3	86.2	87.8	88.0	86.7	87.7	0.58
53	83.0[b]	90.3[a]	86.3	85.9	87.7	86.0	86.5	87.3	0.58
54	81.3[b]	89.4[a]	85.9	84.8	85.1	86.2	84.6	85.1	0.64
55	82.2[b]	89.0[a]	85.4	84.5	86.8	85.4	84.8	86.4	0.61
Average	88.3[b]	93.6[a]	91.2	90.7	90.9	90.8	90.7	91.3	0.34

[a–c] Means within the same row and main effects with different superscripts differ significantly ($P \leq 0.05$).
[1] Total hen day egg production.
[2] Hy–Line CV–20.
[3] Lohmann LSL–Lite.
[4] SEM–Pooled standard error of the mean (N = 90).

TABLE 15.5. Effect of strain, pea inclusion, and lysine level on egg weight (g)

Age (wk)	Strain			Pea inclusion (g/kg)				Lysine intake (mg/h/d)				SEM[3]
	A[1]	B[2]	*P* value	0	150	300	*P* value	700	780	860	*P* value	
22	48.6[b]	51.4[a]	< 0.01	50.3	49.8	49.9	NS	49.9	50.1	50.0	NS	0.204
26	52.6[b]	56.3[a]	< 0.01	54.2[b]	54.8[a]	54.4[ab]	0.032	54.1[b]	54.4[b]	54.9[a]	< 0.01	0.219
30	54.7[b]	58.0[a]	< 0.01	56.1	56.3	56.5	NS	55.8[b]	56.4[a]	56.8[a]	< 0.01	0.212
34	56.4[b]	58.8[a]	< 0.01	56.9[b]	57.7[a]	58.1[a]	< 0.01*	56.5[c]	57.8[b]	58.4[a]	< 0.01	0.199
38	58.6[b]	59.6[a]	< 0.01	58.4[c]	59.2[b]	59.8[a]	< 0.01*	58.2[c]	59.3[b]	59.9[a]	< 0.01*	0.170
42	60.1	59.9	NS	59.5[b]	60.1[ab]	60.3[a]	0.036	58.5[c]	60.4[b]	61.1[a]	< 0.01*	0.189
46	61.2[a]	60.2[b]	< 0.01	59.8[c]	60.7[b]	61.7[a]	< 0.01*	59.4[c]	61.0[b]	61.7[a]	< 0.01*	0.205
50	62.7[a]	61.1[b]	< 0.01	60.8[c]	62.1[b]	62.8[a]	< 0.01*	60.7[c]	62.0[b]	62.9[a]	< 0.01*	0.211
54	62.8[a]	61.6[b]	< 0.01	61.4[c]	62.2[b]	63.0[a]	< 0.01*	61.0[c]	62.2[b]	63.4[a]	< 0.01*	0.205
Average	57.5[b]	58.5[a]	< 0.01	57.5[b]	58.1[a]	58.5[a]	< 0.01*	57.1[c]	58.2[b]	58.8[a]	< 0.01*	0.140

[a–c] Means within the same row and main effects with different superscripts differ significantly ($P \leq 0.05$)
[1] Hy–Line CV–20.
[2] Lohmann LSL–Lite.
[3] SEM–Pooled standard error of the mean (N = 90).
*= Linear regression with $P \leq 0.05$.

TABLE 15.6. Effect of strain, pea inclusion, and lysine level on egg–specific gravity

Age (wk)	Strain		Pea inclusion (g/kg)			Lysine intake (mg/h/d)			SEM[3]
	A[1]	B[2]	0	150	300	700	780	860	
22	1.086[b]	1.092[a]	1.089	1.089	1.089	1.089	1.089	1.089	0.0003
26	1.082[b]	1.088[a]	1.085	1.085	1.085	1.085	1.085	1.084	0.0003
30	1.080[b]	1.085[a]	1.082	1.082	1.082	1.082	1.082	1.082	0.0003
34	1.081[b]	1.085[a]	1.083	1.083	1.083	1.083	1.083	1.083	0.0003
38	1.080[b]	1.083[a]	1.082	1.082	1.081	1.082	1.081	1.081	0.0002
42	1.08[b]	1.083[a]	1.082	1.082	1.082	1.082	1.082	1.082	0.0002
46	1.080[b]	1.082[a]	1.081	1.081	1.081	1.081	1.081	1.081	0.0002
50	1.080[b]	1.082[a]	1.081	1.081	1.081	1.081	1.081	1.081	0.0002
54	1.078[b]	1.080[a]	1.079	1.079	1.078	1.079	1.079	1.078	0.0002
Average	1.081[b]	1.084[a]	1.083	1.083	1.082	1.083	1.083	1.082	0.0002

[a–c] Means within the same row and main effects with different superscripts differ significantly ($P \leq 0.05$)
[1] Hy–Line CV–20.
[2] Lohmann LSL–Lite.
[3] SEM–Pooled standard error of the mean (N = 90).

TABLE 15.7. Effect of strain, pea inclusion, and lysine level on TFEM[1] (g/g)

Age (wk)	Strain			Pea inclusion (g/kg)				Lysine intake (mg/h/d)				SEM[4]
	A[2]	B[3]	*P* value	0	150	300	*P* value	700	780	860	*P* value	
22	2.14	2.17	NS	2.10[b]	2.18[a]	2.20[a]	0.002	2.17	2.14	2.16	NS	0.012
26	1.88[b]	1.97[a]	< 0.01	1.93[ab]	1.91[b]	1.94[a]	0.042	1.94[a]	1.92[ab]	1.90[b]	0.008	0.007
30	1.87[b]	1.97[a]	< 0.01	1.92	1.91	1.93	NS	1.95[a]	1.91[b]	1.89[b]	< 0.01	0.008
34	1.95[b]	2.05[a]	< 0.01	2.01	2.00	2.00	NS	2.05[a]	1.98[b]	1.97[b]	< 0.01	0.009
38	1.91[b]	1.98[a]	< 0.01	1.96	1.94	1.94	NS	1.99[a]	1.94[b]	1.90[c]	< 0.01	0.008
42	1.95[b]	2.01[a]	< 0.01	1.98	1.98	1.98	NS	2.04[a]	1.98[b]	1.92[c]	< 0.01	0.010
46	1.98[b]	2.05[a]	< 0.01	2.04[a]	2.01[ab]	1.99[b]	0.030	2.08[a]	2.00[b]	1.96[c]	< 0.01	0.010
50	2.01[b]	2.10[a]	< 0.01	2.09[a]	2.04[b]	2.04[b]	0.021	2.11[a]	2.05[b]	2.01[b]	< 0.01	0.010
54	2.01[b]	2.05	0.023	2.04[a]	2.05[a]	2.00[b]	0.022	2.07[a]	2.04[a]	1.97[b]	< 0.01	0.009
Average	1.97[b]	2.04[a]	< 0.01	2.01	2.00	2.00	NS	2.05[a]	2.00[b]	1.97[c]	< 0.01	0.007

[a–c] Means within the same row and main effects with different superscripts differ significantly ($P \leq 0.05$).
[1] Total feed per egg mass.
[2] Hy–Line CV–20.
[3] Lohmann LSL–Lite.
[4] SEM–Pooled standard error of the mean (N = 90).

TABLE 15.8. Effect of strain, pea inclusion, and lysine level on TFDE[1] (kg/dozen)

Age (wk)	Strain			Pea inclusion (g/kg)				Lysine intake (mg/h/d)				SEM[4]
	A[2]	B[3]	*P* value	0	150	300	*P* value	700	780	860	*P* value	
22	1.25[b]	1.34[a]	< 0.01	1.27[b]	1.30[a]	1.31[a]	< 0.01	1.30	1.29	1.29	NS	0.007
26	1.19[b]	1.33[a]	< 0.01	1.25	1.25	1.26	NS	1.26	1.26	1.25	NS	0.008
30	1.23[b]	1.37[a]	< 0.01	1.29	1.29	1.31	NS	1.31	1.30	1.29	NS	0.009
34	1.32[b]	1.45[a]	< 0.01	1.37	1.38	1.40	NS	1.39	1.37	1.38	NS	0.008
38	1.34[b]	1.42[a]	< 0.01	1.37	1.38	1.39	NS	1.39	1.38	1.37	NS	0.006
42	1.41[b]	1.44[a]	< 0.01	1.41	1.43	1.43	NS	1.43	1.43	1.41	NS	0.005
46	1.45[b]	1.48[a]	0.025	1.46	1.46	1.47	NS	1.48	1.47	1.45	NS	0.005
50	1.52	1.54	NS	1.52	1.52	1.54	NS	1.54	1.52	1.52	NS	0.005
54	1.52	1.51	NS	1.50	1.53	1.51	NS	1.52	1.52	1.50	NS	0.005
Average	1.36[b]	1.43[a]	< 0.01	1.38	1.39	1.40	NS	1.40	1.39	1.39	NS	0.005

[a–c] Means within the same row and main effects with different superscripts differ significantly ($P \leq 0.05$).
[1] Total feed per dozen of egg.
[2] Hy–Line CV–20.
[3] Lohmann LSL–Lite.
[4] SEM–Pooled standard error of the mean (N = 90).